U.S. DEPARTMENT OF COMMERCE
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PB-263 030

Proceedings of Workshop on Microorganisms in Urban Stormwater Held at Edison, New Jersey, on March 24, 1975

Municipal Environmental Research Lab, Edison, N J

Prepared for

Environmental Protection Agency, Cincinnati, Ohio Office of Research and Development

Nov 76

PB 263 030

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Environmental Protection Technology Series

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-76-244	2.	3. RECIPIENT'S ACCESSION NO.

4. TITLE AND SUBTITLE PROCEEDINGS OF WORKSHOP ON MICROORGANISMS IN URBAN STORMWATER	5. REPORT DATE November 1976 (Issuing Date) 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Richard Field, Vincent P. Olivieri, Ernst M. Davis, James E. Smith, Edwin C. Tifft, Jr.	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Storm and Combined Sewer Section Wastewater Research Division Municipal Environmental Research Laboratory (Cincinnati) Edison, New Jersey 08817	10. PROGRAM ELEMENT NO. 1BC611 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	13. TYPE OF REPORT AND PERIOD COVERED Workshop Proceedings 14. SPONSORING AGENCY CODE EPA-ORD

15. SUPPLEMENTARY NOTES

Workshop Proceedings based on EPA Grants S802433, R802709 and S802400.
P.O. Richard Field (201) 548-3347 X503 (8-342-7503)

16. ABSTRACT

This workshop was held on March 29, 1975 at the USEPA Office, Edison, New Jersey. The aim was to exchange information obtained from USEPA Office of Research and Development, Storm and Combined Sewer Program sponsored projects so as to foster a better understanding of microorganisms in urban storm runoff and combined sewer overflow.

Workshop emphasis was placed on the following aspects:

a. Procedures for pathogenic microorganism assays

b. Relationship between pathogenic and coliform group microorganisms

c. Disinfection and aftergrowth of microorganisms

d. Viruses in stormwater

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Waste water, Sewage, Microorganisms, Viruses, Coliform bacteria, Disinfection, Contaminants, Waste treatment, Water pollution, Water treatment, Bacteria, Salmonella, Water quality, Shigella, Streptococcus, Storm sewers, Pseudomonas, Surface water runoff, Runoff	Pathogenic microorganisms /coliform bacteria relationship, Combined sewer overflows, Wastewater treatment, Water pollution sources, Storm runoff	13B

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 132
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE [illegible]

☆USGPO: 1977 — 757-056/5441 Region 5-II

EPA-600/2-76-244
November 1976

PROCEEDINGS OF WORKSHOP ON MICROORGANISMS IN URBAN STORMWATER

Edison, New Jersey
March 24, 1975

by

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

It is with these objectives in mind that the results of this workshop on microorganisms in urban stormwater are published.

Francis T. Mayo
Director
Municipal Environmental Research
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ABSTRACT

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ACKNOWLEDGMENTS

Mr. Chi-Yuan Fan, previously staff engineer of the Storm and Combined Sewer Section and now managing engineer with Envirosphere, Inc., New York, NY deserves special recognition for devoted effort in helping to organize the workshop.

Aside from the lecturers, the other workshop participants (listed on pages vi and vii) are thanked for their contributions in discussions and their reviews of various facets of the projects involved with the workshop. These efforts have subsequently helped to better direct the EPA Stormwater Program.

Sincere thanks are given to Mrs. Elizabeth H. Mohary for her most valuable assistance in organizing these proceedings and in typing.

Richard Field
Workshop Director

MICROORGANISMS IN URBAN STORMWATER --
A U.S. ENVIRONMENTAL PROTECTION AGENCY PROGRAM OVERVIEW

by

Richard Field

Urban storm discharges whether they be combined with municipal sewage or separate stormwater occur on an intermittent and random basis. After rainfall, these flows exhibit highly varying patterns in both pollutant and microorganism quality and hydraulic quantity over short periods of time. A sewer or channel can flow from completely dry to a 1000 times the nearly steady-state flow conditions of sanitary wastewater. Temperature fluctuation is also much greater for stormwater than for sanitary sewers which points to the possible importance of temperature in addition to the usual time and dosage disinfection control parameters. Consequently, it has been difficult to adapt existing analytical and disinfection methods to microorganisms in storm flow.

Three basic needs have arisin for the control of microorganisms in storm flow:

First, to know its microorganism pathogenetic quality and the pathogens' relationships to other indicator microorganisms. The pathogen indicators, i.e. total coliform and sometimes fecal coliform and fecal streptococcus, are conventionally used in stormwater studies. These parameters have been adopted almost blindly out of their routine use for sanitary sewage analysis into a new consideration -- urban stormwater. Resulting analytical data can misrepresent the actual harmful potential or pathogenic microorganism contents of the storm flow dealt with. Extremely high coliform counts can come from sources such as soils and animals, other than humans. Unnecessary and costly disinfection facilities could result or possibly underdesigned facilities could also result;

The second need is to develop high-rate disinfection systems to save on large tankage or dosage requirements for the high storm flow rates encountered; and

Third, to develop disinfection facility design and operation techniques for the highly varying qualitative and quantitative character of the storm-generated inflows.

I do not want to give the impression that methods for a truer analysis of pathogen quality, and more rapid and better operation of disinfection facilities are no longer needed for domestic waste flows -- for we all know that they are. What I am implying is that the need is more apparent for storm flows because the dry-weather municipal waste flows are nearly steady-state, more historical data is known for them, and they are less intense as compared to wet-weather flows.

Over the past seven or eight years our urban stormwater research, development, and demonstration program has instituted and implemented a number of projects wholly or partially dedicated to the determination or disinfection of microorganisms in urban stormwater and combined sewer overflows; and some impressive results have been obtained. Some of these results will be discussed here today by others.

Previous stormwater program ventures in the area of microorganisms and disinfection included:

First: The characterization of indicator microorganisms, where it was found that coliform counts ranged from the order of magnitude found in domestic wastewater to a few orders less; however, in almost all cases, disinfection would be required based on the present standards recommended.
Second: On-site disinfectant generation by raw materials' batching[1] and electrolysis[2] in order to provide the large and intermittantly unpredictable quantities of disinfectant required economically; and
Third: High-rate disinfection with the help of more rapid oxidants (e.g. chlorine dioxide[3] and ozone[4,5]), two stage "booster" disinfectant dosing,[3] and by imparting greater turbulence by static and mechanical means.[4-6] We have made a special point not to just arbitrarily increase dosages without regard for the potential residual toxicity problems. In this regard, chlorine dioxide which does not form chloramines, and ozone with its relatively short half-life, may also be the better choice.

In our study in Philadelphia,[4-6] it was found that by using principles from the field of particle flocculation for potable water treatment, a disinfection tank with closely spaced corrugated baffles could be designed to provide adequate coliform kills in under two minutes contact time with less than three mg/l chlorine. The closely spaced baffle arrangement also insures plug flow for optimum utilization of the tank volume. Four mg/l of ozone with a contact time of 3.3 minutes was also shown to be effective in a side-by-side comparison.[4,5]

At present, ozone does not appear as suitable as chlorine due to comparative costs. This is most likely caused by the power requirements for on-site ozone generation. However, the use of ozone should not be overlooked since the cost gap may be closing and there may be an economically defined need for on-site chlorine generation when it comes to storm flow application (as previously discussed). Other advantages of ozone are that it -- (a) is a more powerful oxidant reacting more vigorously with organics, (b) destructs phenols rather than forming obnoxious chlorophenols, (c) may be a better viricide, and (d) by the method of air application increases effluent dissolved oxygen.

Again, some of the ongoing research that will be discussed today covers the areas of primary need which include characterization, high-rate disinfection, and adequate operational control.

In 1962, the ASCE committee on Public Health Activities examined the relationship between coliform concentration in bathing waters and the occurrence of enteric diseases among bathers. It was stated that "there is a remarkable lack of evidence that high concentrations of coliform organisms in recreational waters are accompanied by enteric disease cases." In a recent EPA statement on proposed wastewater disinfection policy mention was made to eliminate coliform limitations from secondary treatment requirements and to establish disinfection requirements case-by-case according to water quality and public health needs. The benefits from microorganism kill should be weighed against environmental risks from toxic residuals.

The evidence points to a strong need for more effective analysis and disinfection of microorganisms. Perhaps we all agree that the only conceivable way to go about this is by the interrelation with carefully planned epidemiological studies; however, we may also agree that in the interim the development of more meaningful relationships between levels of the customary indicators to pathogenic microorganisms and direct pathogen examination could lead us in the right direction towards an ultimate cure.

The principal purpose of today's workshop is for an exchange of project information and data, and to establish liasons between principal investigators on three EPA sponsored research projects for the mutual benefit of their respective investigations. The first speaker will be Dr. Vincent Olivieri from the Johns Hopkins' School of Public Health and Hygiene. His project, "Microorganisms in Stormwater," began in May 1974 and is slated for completion in December 1975. The objective of Dr. Olivieri's study (EPA Research Grant No. R802709) is to provide basic information for the conduct of more scientific evaluations on the health hazard potential from bacteria in urban storm flows.

The next speaker will be Dr. Ernst Davis from the University of Texas' School of Public Health. His study is under an EPA project dealing with total water resources for a planned community near Houston (EPA Research Grant No. S802433). Dr. Davis will present data from his study to determine disinfection requirements for the community. Indicator and pathogen analyses have been conducted on stream and runoff samples. Eventually he hopes to demonstrate how urbanization influences bacterial quality as the community develops. The project began during the summer of 1973 and is due to be completed the summer of 1976. I would like to mention that a colleague of Dr. Davis' (also involved in the project) is performing fish assays to analyze the after-effects of chlorine and ozone.

Our third speaker will be Dr. James Smith of Syracuse University who will discuss viral recovery and inactivation as a result of his experiences from two stormwater program projects, the previously mentioned Johns Hopkins study in Baltimore and a demonstration project with Onondaga County in Syracuse, New York (EPA Demonstration Grant No. S802400).

Lastly, Dr. Ted Tifft of O'Brien & Gere, Consulting Engineers, will present interesting results from his work on disinfection of combined sewer overflows with chlorine and chlorine dioxide from the Onondaga study. The project started in July 1972 and is due to be completed the end of 1976.

Each speaker will have 45 minutes for his discussion which includes 30 minutes for presentation and 15 minutes for questions and answers. A detailed discussion is scheduled after all presentations are completed. With the assistance of Frank Condon, who has a thorough familiarity of stormwater applications, and Cecil Chambers, Ed Geldrich, Gerald Berg, Irwin Katz, Cornelius Kruse and Fran Brezinski, who are world renowned experts in the field of waterborne microorganisms and disinfection, I am hopeful that the discussion periods will be worthwhile.

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EXPERIENCE ON THE ASSAY OF MICROORGANISMS IN URBAN RUNOFF

Workshop on Microorganisms in Stormwater

by

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INTRODUCTION

The high levels in urban runoff of microorganisms indicative of fecal contamination has been well documented in the literature (Weibel *et al.* (1), Geldreich *et al.* (2), Evans *et al.* (3), and Burm *et al.* (4)). However, little information is available on the level of pathogenic microorganisms in stormwater runoff in urban areas. The specific objectives of this project are to obtain information on the levels of pathogenic microorganisms in stormwater and evaluate the relationships between indicator microorganisms and pathogens.

The evaluation of methods of detection and enumeration is a fundamental prerequisite for obtaining reliable results. Techniques and procedures employed in the laboratory for a given set of samples may yield poor results in another laboratory for a different sample. The microbial flora can vary significantly in water samples depending on environmental conditions and sources of contamination. The types and levels of interfering microorganisms become an important factor and will vary with the procedure employed. Techniques, methods and culture media developed for microbial assays of clinical specimens may yield poor results when applied to water samples where different interfering microorganisms are present. No one method for the detection and enumeration of a particular group of microorganism can be universally employed.

The early phase of the current project involved the evaluation of methods of detection and enumeration of the microorganisms to be assayed. Techniques and culture procedures described in *Standard Methods for the Analysis of Water and Wastewater* (5) and in recent literature were evaluated. Where similar results were obtained, the cultural procedures in *Standard Methods* were chosen. Where the procedures in *Standard Methods* were found lacking, alternative methods were developed. The final selection of techniques, methods and procedures was based on simultaneous analysis of water samples in our laboratory and information in the literature. Multiple tube dilution procedures which permitted a calculation of a most probable number (MPN) were generally favored because of the wide variability in the chemical and physical characteristics (particularly solids) expected in the samples from the urban water courses.

Grab samples were collected in the Baltimore metropolitan area and assayed for the levels of total coliform, fecal coliform, fecal streptococci, *Salmonella, Shigella, Pseudomonas aeruginosa, Staphlococcus aureus,* and animal viruses. Viral assays were performed by Dr. James Smith of Syracuse University. Two sets of samples were taken in the study. Background samples were collected from three urban streams and raw sewage on a routine schedule regardless of rainfall. Recently an upland water source has been added. These samples are designated background samples. Six samples were collected during storms at outfalls within the Baltimore metropolitan area to provide information on the levels of microorganisms in runoff. Both combined and separate sewers were sampled. Sites were selected to provide a cross section of drainage areas found in an urban environment.

METHODS

DIFFERENTIAL TESTS

The following tests were employed to provide information for the tentative identification of isolates obtained from the microbial assays. A spot inoculation procedure on differential agar plates was employed where possible. Isolates were transferred with sterile toothpicks to a 35 or 50 place grid pattern on 100 mm Petri dishes containing the appropriate agar medium.

Phenylalanine deaminase

Isolates were spotted on phenylalanine agar (Difco) and incubated for 24 hours at 37°C. Phenylalanine deaminase activity was indicated by a green zone around the colony after flooding the plate with a 0.5M ferric chloride solution (6).

Oxidase

Oxidase production was determined for isolates spotted on tech agar (Baltimore Biological Laboratory) after incubation at 37°C for 18 to 24 hours. The tech agar plate was flooded with a 1% p-amino dimethylaniline monohydrochloride. Oxidase positive colonies turn pink within one minute.

Triple Sugar Iron Agar (TSI)

TSI slants were prepared in 13 x 100 mm culture tubes. Stab-streaks were prepared for each isolate and incubated at 37°C. pH changes in the butt and slant were observed after 18 to 24 hours. Hydrogen sulfide production was observed after 48 hours incubation.

Lysine Iron Agar (LIA)

LIA slants were prepared as above for TSI and used in conjunction with TSI as recommended by Edwards and Ewing (7).

Malonate Utilfzation

Malonate utilization was determined in malonate broth in 13 x 100 mm culture tubes according to the procedures described in Edwards and Ewing (7). The change in indicator from green to prussian blue indicated malonate utilization.

Lysine Decarboxylase

Lysine decarboxylase activity was determined by the method of Moeller described in Edwards and Ewing (7). Decarboxylase base medium with and without lysine was inoculated from fresh agar slant cultures, overlaid with sterile mineral oil and incubated at 37°C. The cultures were examined daily for four days. Lysine decarboxylase activity was indicated by the change from yellow to violet in the tubes containing lysine. Control tubes without lysine remained yellow.

Salmonella Polyvalent Antisera

Isolates that yielded typical or suspicious *Salmonella* reactions were submitted to preliminary serological examination with *Salmonella* O antiserum Poly A-I including Vi (Difco) by slide agglutination. Positive tests are indicated by rapid complete agglutination of the bacterial cells.

Urease

Urease activity was determined by the spot inoculation procedure on plates prepared with Christensen's urea agar (7). Urease activity was indicated by the formation of a red zone around the colony after 2 to 6 hours of incubation. Delayed reactions could not be observed by this procedure.

Citrate Utilization

Citrate utilization was determined by the spot inoculation procedure on plates containing Simmons citrate agar. Colony formation and color change from green to blue in the medium indicated citrate utilization (7).

Coagulase

The elaboration of coagulase was determined by the plate method described by Esber and Faulconer (8). Isolates were transferred to coagulase agar (Difco) and incubated overnight. *Staph. aureus* stock cultures were included for each fresh batch of media. Coagulase positive and mannitol positive strains yield a yellow opaque zone around the colony. The plate procedure was evaluated by comparing the elaboration of coagulase by the conventional tube procedure for 150 isolates. 100% agreement was observed for the two procedures.

DNase

DNase production was performed by the method of Streitfeld *et al.* (9). Colonies were transferred to DNase test agar (Baltimore Biological Laboratories) and incubated overnight. After incubation the plate was flooded with 0.1% toluidine blue. DNase production is indicated by a rose pink zone around the colonies.

Lipovitellenin-Lipase

Lipovitellenin-Lipase was determined on Lipovitellenin-salt-mannitol agar (LSM) (10). Opaque zones around the colonies indicated lipovitellenin-lipase activity.

Mannitol Fermentation

Mannitol fermentation was observed on LSM and coagulase agar. Yellow zone around the colonies was taken as positive mannitol fermentation.

Anaerobic Glucose Fermentation

Representative isolates that yielded typical *Staph. aureus* reactions were further tested for the ability to ferment glucose anaerobically by the method described by Evans and Kloos (11) to separate any possible soil micrococci. An overnight culture on brain heart infusion (BHI) broth at 37°C was used to inoculate tubes of Brewer's fluid thioglycolate medium (BTM) containing 0.35% agar. Growth throughout the tube after 24 hours was indicative of anaerobic glucose fermentation.

Gram Stain

Gram stains were prepared according to the procedures described in *Standard Methods* (5).

Gelatin Liquefaction

Gelatin liquefaction was determined on BHI containing 120 g/liter of gelatin. Isolates were spot inoculated on plates and incubated at 10°C for five days. Liquid zones around colonies indicated gelatin liquefaction.

Mannitol and Arabinose Fermentation

Mannitol and arabinose fermentation were determined separately on plates of phenol red carbohydrate fermentation medium containing 10 g/liter of each sugar and 2% agar. Plates were incubated at 37°C for 18 hours. Yellow zones around the colonies indicated fermentation.

Growth in Bile

Growth in 40% bile and 6.5% NaCl was determined on separate BHI agar plates containing 40 g/liter oxgall and 65 g/liter NaCl. Colony formation at the point of inoculation after 48 hours incubation at 35°C was considered an indication of growth.

Growth at 45°C and 10°C

Growth at 45°C and 10°C was determined on BHI agar after incubation for 48 hours and five days, respectively. Colony formation at the point of inoculation was considered an indication of growth.

Catalase

Catalase activity was determined for isolates from the fecal streptococci assay be adding a drop of 3% hydrogen peroxide to each colony on the BHI agar replicate control plate after incubation at 37°C for 24 hours. Gas bubbles indicated catalase activity. Catalase activity for *Staphylococci* isolates was determined by transferring a portion of the colony to 3% hydrogen peroxide. Evolution of gas indicated a positive catalase.

Starch Hydrolysis

Starch hydrolysis was determined on nutrient agar containing 10 g/liter of soluble starch and 8 g/liter NaCl. After incubation at 37°C for 48 hours each plate was flooded with Gram's iodine. Clear zones around the colony indicated starch hydrolysis.

Casein Hydrolysis

Casein hydrolysis was determined on plates of skim milk agar (10 g/liter skim milk and 15 g/liter agar) after incubation for three to five days at 37°C (12). Hydrolysis of casein was indicated by the formation of clear zones around the colony. *Pseudomonas* isolates were observed for fluorescence when exposed to ultra-violet light.

Growth on Acetamide

Growth on acetamide was rechecked by spotting isolates on acetamide agar (5) and incubated for 48 hours. Pink to red colonies with blue fluorescence when exposed to ultra-violet light were considered positive.

Growth at 42°C

Acetamide and oxidase positive isolates were transfered to Drake's medium #10 for evaluation of growth at 42°C (13) after 48 hours incubation. Growth was indicated by turbidity with blue green fluorescence.

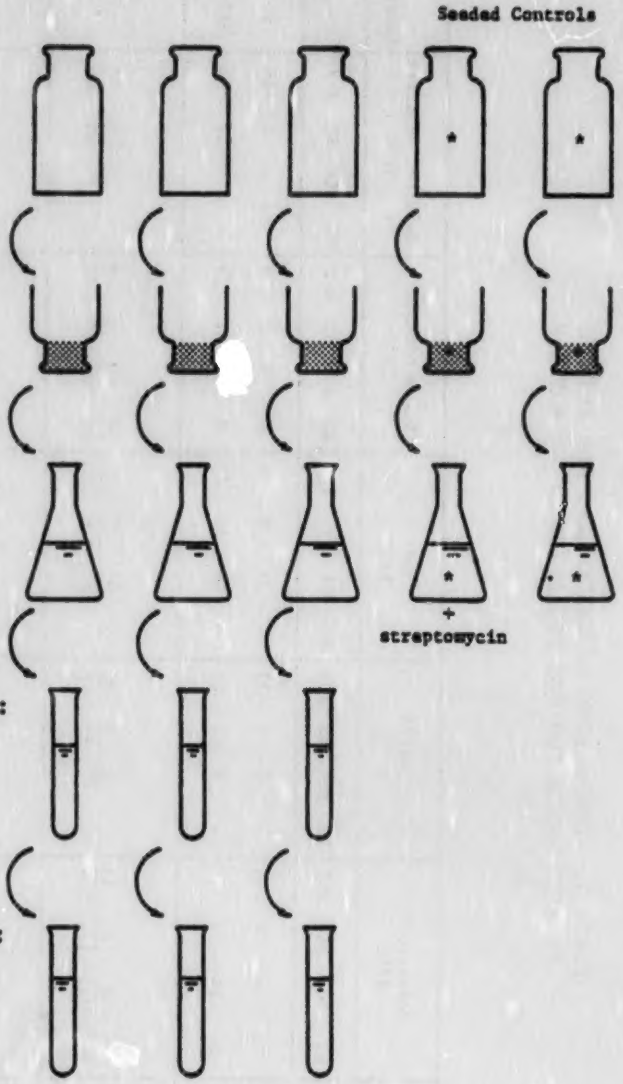
CULTURE EVALUATION AND PROCEDURES

SALMONELLA sp.

A multiple concentration and enrichment procedure was employed to permit the calculation of a MPN *Salmonella* for each sample. The procedure was similar to that described by Kenner and Clark (14) except that diatomaceous earth was used for concentration. The multiple concentration and enrichment procedure is shown in schematic form in Figure 1. A three replicate MPN plus three seeded controls were employed to permit an evaluation of the recovery procedures for each sample. A laboratory strain, *Salmonella typhimurium* SB558, resistant to 1000 µg/ml of streptomycin was used as the seed *Salmonella*. The *Salmonella* seed was prepared from cultures stored at -40°C in 23% glycerol. The low temperature glycerol storage provided a readily available test organism at a known density. The three seeded controls evaluated sample toxicity, diatomaceous earth concentration and the overall recovery procedure. A 100-fold dilution of the *Salmonella* seed stock was prepared for each sample to evaluate sample toxicity. The mixture was maintained at room temperature for the duration of the sample processing period and plated on brain heart infusion (BHI) agar containing 1000 µg/ml streptomycin. Samples were considered toxic when more than 90% inactivation was observed. The diatomaceous earth concentration was evaluated by the addition of 5 to 50 *Salmonella* to a replicate of each sample filtered. The diatomaceous earth plug was transferred to enrichment medium containing 1000 µg/ml of streptomycin. The recovery of streptomycin-resistant *Salmonella* was considered positive concentration on the diatomaceous earth. The overall concentration and culture procedure was evaluated with an additional replicate of each sample prepared as above. The streptomycin, however, was omitted from the enrichment medium. Isolates obtained from the primary plates were tested for streptomycin resistance. The isolation of streptomycin-resistant *Salmonella* indicated a positive recovery.

During the initial portion of the study various combinations of enrichment media, enrichment temperatures and primary plating media were evaluated using 22 samples prepared with a multiple concentration on diatomaceous earth and multiple enrichment. The results are given in Table 1. The enrichment media and temperatures evaluated were selenite broth at 37°C, selenite broth at 41°C, GN broth at 37°C, tetrathionate broth at 37°C and dulcitol selenite broth at 40°C. The primary plating media employed were bismuth sulfite (BS), brilliant green agar (BGA), Salmonella Shigella agar (SS) and xylose lysine desoxycholate agar (XLD). A total of 527 isolates were tested during this phase of the investigation. The elevated temperature enrichments consistently yielded higher numbers of *Salmonella* isolates for primary plating media. The final choice was dulcitol selenite at 40°C coupled with primary plating on XLD similar to the procedure reported by Kenner and Clark (14). Identification of the members of the genus *Salmonella* was performed according to the schematic given in Figure 2. Typical *Salmonella* colonies on XLD (pink colonies with black centers or pink colonies) were screened for phenylalanine deaminase and oxidase activities. Enrichment cultures that did not yield typical *Salmonella* colonies were restreaked at 48 or 72 hours on XLD and subsequent typical colonies were handled as above.

Figure 1. Schematic - Multiple concentration and enrichment for the MPN determination of *Salmonella*



Sample:
3 or 5 replicates and seeded control.

Concentration:
diatomaceous earth.

Multiple Enrichment:
(1) 10 g filter plug suspended in 1 x enrichment medium to 50 ml.
Effective volume:
0.9 x sample.

(2) 10-fold dilution:
5.0 ml of (1) to 5.0 ml 1 x enrichment medium.
Effective volume:
0.09 x sample.

(3) 10-fold dilution:
1.0 ml of (2) to 9.0 ml 1 x enrichment medium.
Effective volume:
0.009 x sample.

(4) Subsequent 10-fold dilutions if necessary. 1.0 ml final dilution removed to waste to maintain serial dilution.

* Seed - 10 to 50 organisms/sample vol. of streptomycin resistant *Salmonella typhimurium* SB558

Table 1. COMPARISON OF ENRICHMENT AND PRIMARY PLATING MEDIA FOR THE ISOLATION OF *SALMONELLA*

Enrichment Temp.	Selenite F 37°C				Selenite 41°C				GN 37°C				Tetrathionate 37°C				Dulcitol-Selenite 40°C				Totals
Primary Plating	BS	BGA	SS	XLD	BS	BGA	SS	XLD	BS	BGA	SS	XLD	BS	BGA	SS	XLD	BS	BGA	SS	XLD	
# Positive	1	0	2	11	-	0	1	37	-	0	0	1	0	0	0	0	-	1	8	28	90
# Isolates	83	16	36	12	-	9	21	67	-	16	59	45	2	14	32	33	-	9	32	41	527
Primary Plate $\frac{\text{\# Positive}}{\text{\# Isolated on Enrichment}}$	$\frac{1}{147}$	$\frac{0}{147}$	$\frac{2}{147}$	$\frac{11}{147}$	-	$\frac{0}{97}$	$\frac{1}{97}$	$\frac{37}{97}$	-	$\frac{0}{120}$	$\frac{0}{120}$	$\frac{1}{120}$	$\frac{0}{81}$	$\frac{0}{81}$	$\frac{0}{81}$	$\frac{0}{81}$	-	$\frac{1}{82}$	$\frac{8}{82}$	$\frac{28}{82}$	
Enrichment $\frac{\text{\# Positive}}{\text{\# Isolated on Enrichment}}$	$\frac{14}{147}$				$\frac{38}{97}$				$\frac{1}{120}$				$\frac{0}{81}$				$\frac{37}{82}$				
$\frac{\text{\# Positive}}{\text{Total \# Positive}}$	$\frac{14}{90}$				$\frac{38}{90}$				$\frac{1}{90}$				$\frac{0}{90}$				$\frac{37}{90}$				

Note (+) Data based on 22 samples (7 raw sewage and 15 urban streams) prepared with a multiple concentration on celite and multiple enrichment

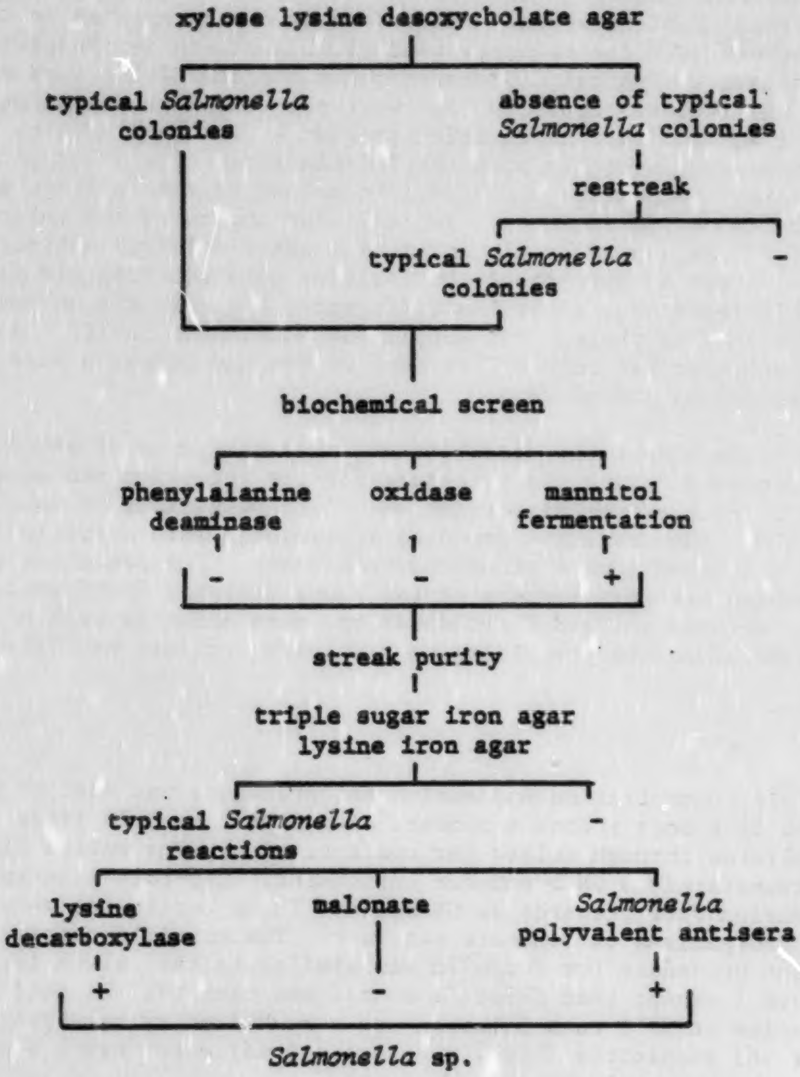


Figure 2. Schematic - Isolation and identification of *Salmonella* sp.

xylose lysine desoxycholate agar
typical *Salmonella* colonies
absence of typical *Salmonella* colonies
restreak
typical *Salmonella* colonies
-
biochemical screen
phenylalanine deaminase
oxidase
mannitol fermentation
-
-
+
streak purity
triple sugar iron agar
lysine iron agar
typical *Salmonella* reactions
-
lysine decarboxylase
malonate
Salmonella polyvalent antisera
+
-
+
Salmonella sp.

Phenylalanine deaminase negative and oxidase negative isolates were streak purified again and transferred to triple sugar iron agar (TSI) and lysine iron agar (LIA). Typical *Salmonella* was tested for malonate utilization and lysine decarboxylase activity and submitted to a serological examination with polyvalent antisera A-I (including Vi).

Phenylalanine deaminase and oxidase were employed to eliminate the most probable interferences from members of the genus *Proteus, Providencia* and *Pseudomonas* and, thereby, minimize the number of isolates submitted to further tests. Table 2 shows a comparison of the phenylalanine deaminase and oxidase screen with the commonly used TSI-LIA screen for suspicious *Salmonella* isolates. Typical *Salmonella* reactions on TSI-LIA were obtained for 92.8% of the isolates from XLD that were phenylalanine deaminase and oxidase negative. The spot inoculation procedure for phenylalanine deaminase and oxidase compares favorably with the TSI-LIA screen for *Salmonella* and provides a rapid, inexpensive and effective method to screen large numbers of suspicious *Salmonella* isolates. Table 2 also indicated the relative efficiency of XLD as a primary plating medium after dulcitol selenite enrichment at 40°C. About 53 percent of the isolates obtained from XLD yielded typical TSI-LIA reactions. A marked difference, however, can be seen for the two types of colony morphology for suspicious *Salmonella* on XLD. 84.5% of the black centered but only 4.3 percent of the red colonies were positive through the screening procedure.

Table 3 shows a tentative identification of the genus of microorganisms commonly encountered during the procedure for the detection and enumeration of *Salmonella*. Each of the microorganisms yield suspicious *Salmonella* colonies on XLD. The tentative grouping of isolates into a particular genus was based on a limited number of biochemical tests. The predominant interfering microorganisms were members of the genus *Proteus, Providencia* and *Pseudomonas*. *Arizona* sp. and *Citrobacter* sp. were about 3% each of the isolates tested after the phenylalanine deaminase, oxidase and TSI-LIA screens.

SHIGELLA sp.

A multiple concentration and enrichment procedure was used to permit a calculation of a most probable number. Two liters to 3.79 liters were initially filtered through celite for concentration. The entire plug of celite was transfered to GN broth for enrichment. Ten-fold dilutions of the celite suspension were prepared in GN broth. Three replicates were run at 10 to 50 microorganisms to evaluate recovery. The multiple concentration and enrichment procedure for *Shigella* was similar to that given for *Salmonella* in Figure 1 except that *Shigella sonnei* was used for the seed control. After incubation at 37°C each dilution was streaked on xylose lysine deoxycholate agar and suspicious *Shigella* colonies (red) were tested biochemically according to the protocol shown in Figure 3.

Eighteen samples of raw sewage and urban streams were assayed according to the above procedure. It should be noted that each sample represents nine attempts to isolate *Shigella* (3 replicates x 3 dilutions). More than

Table 2. COMPARISON OF THE PHENYLALANINE DEAMINASE (ϕ) AND OXIDASE (OX) SCREEN WITH TSI AND LIA REACTIONS FOR THE DIFFERENTIATION OF *SALMONELLA*

Number Typical *Salmonella* Reactions (%)

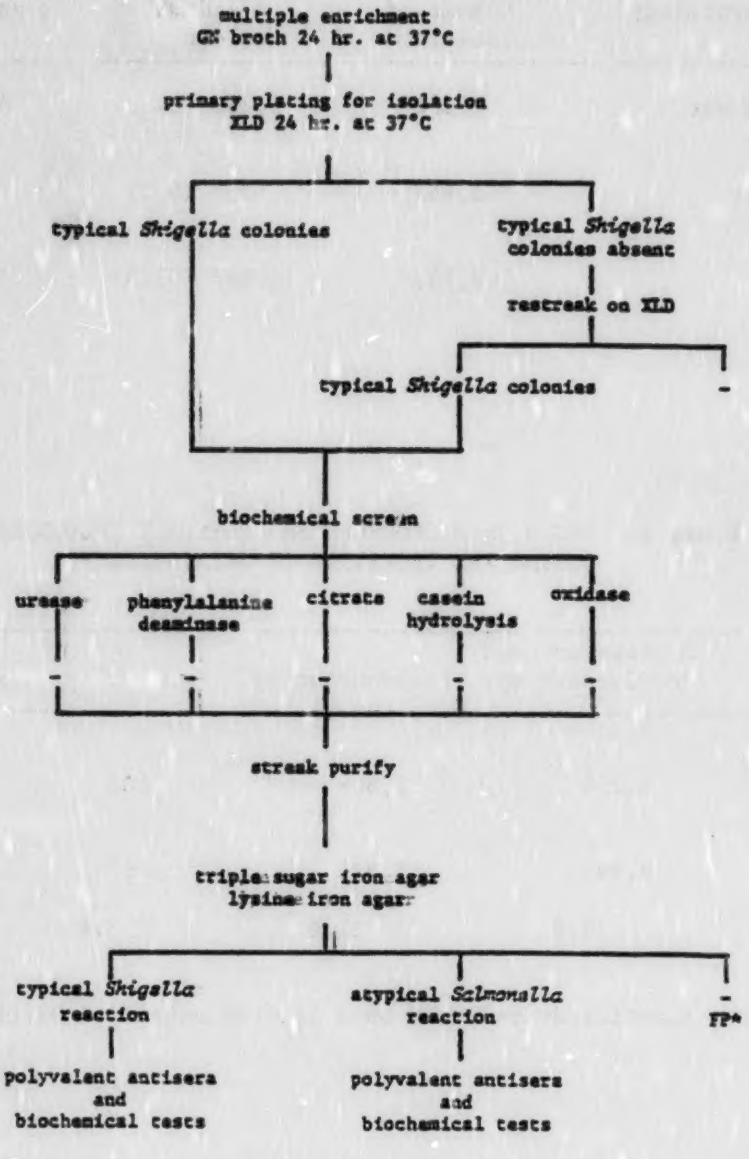
Colony morphology on XLD	Number of isolates	ϕ and OX screen	ϕ and OX followed by TSI-LIA
Black centered colony	6,258	5,286 (84.5)	5,142 (82.2)
Red colony	3,457	288 (8.3)	149 (4.3)
Total	9,715	5,589 (57.5)	5,186 (53.4)

Table 3. GENUS OF MICROORGANISMS COMMONLY ENCOUNTERED DURING THE ISOLATION OF *SALMONELLA* *

	Proteus sp. and *Providencia* sp.	*Pseudomonas* sp.	*Arizona* sp.	*Citrobacter* sp.
Number isolated	1,250	2,809	108	96
Number tested	8,995	8,995	2,967	2,967
%	13.9	31.6	3.6	3.2

* Tentative identification based on a limited number of biochemical tests

Figure 3. Schematic - Isolation and identification of *Shigella* sp.


multiple enrichment
GN broth 24 hr. at 37°C
primary plating for isolation
XLD 24 hr. at 37°C
typical *Shigella* colonies
typical *Shigella* colonies absent
restreak on XLD
typical *Shigella* colonies
-
biochemical screen
urease
phenylalanine deaminase
citrate
casein hydrolysis
oxidase
-
-
-
-
-
streak purify
triple sugar iron agar
lysine iron agar
typical *Shigella* reaction
atypical *Salmonella* reaction
-
FP*
polyvalent antisera and biochemical tests
polyvalent antisera and biochemical tests

---

* FP indicates false positive

1,100 suspicious isolates from XLD were submitted to further biochemical tests. *Shigella* was not found in any of the samples tested nor was the seeded *Shigella* ever recovered. Approximately 10% of the isolates were negative in preliminary biochemical screen indicating possible *Shigella*. One isolate yielded typical reactions for *Shigella* on TSI and LIA but was serotypically negative and hydrolyzed casein. Table 4 is a tentative identification of the major groups of microorganisms isolated. *Pseudomonas* species, *Providencia* species and non-H_2S producing members of the genus *Proteus* yield red colonies on XLD agar indistinguishable from *Shigella*.

Levels of indicator microorganisms and *Salmonella* for the samples negative for *Shigella* are given in Table 5. One would expect to isolate *Shigella* from the raw sewage and streams containing relatively high levels of total and fecal coliforms, fecal streptococci and *Salmonella*. The limitation appears to make the methodology poor.

The celite concentration procedure employed appears to function reasonably well. Table 6 shows the results of control experiments with low level seeded *Shigella* in phosphate-buffered saline. The recovery of *Shigella* on celite is well within the 95% confidence limits for the MPN procedure. The major difficulty appears to be in the enrichment procedures and methodology. *Shigella* does not appear to compete and survive well in the presence of other actively growing microorganisms. Hentges (15) reported the inhibition of *Shigella* when grown with coliforms and *Klebsiella*. He attributed the inactivation of *Shigella* to organic acids produced by the other microorganism and that a pH below 7 enhanced the effect.

Rather than continue the unproductive *Shigella* assays, the effort was directed at providing some information to explain the inability to isolate *Shigella*. Older literature suggests that *Shigella* is a fragile microorganism in the environment. This assumption of fragility is adequately dispelled by the reports of the ability of laboratory cultures of *Shigella* to survive when added to a variety of polluted and non-polluted waters. The absolute survival times vary markedly from study to study dependent on environmental conditions. Laboratory experiments indicate that at 20°C *Shigella* persisted for 12 days in farm pond water (16). McFeters *et al.* (17) reported half time die-off rates of 22.4 hours, 24.5 hours and 26.8 hours for *Shigella dysenteriae*, *Shigella sonnei* and *Shigella flexneri* at 9.5 to 12.5°C in well water. Bartos *et al.* (18) reported recovery of added dysentery bacilli after 22 days in well water and 40 days in well water with coliforms added. Dolivo-Dobrovol'skii and Rossovaskaia (19) reported *Shigella* survivals from 30 minutes to four days and indicated that aeration markedly reduced survival time. Under conditions of extreme cold (-45°C) *Shigella* persisted 145 days in feces, 135 days in soil and 47 days in frozen river water (20). Although conditions vary, *Shigella* does not appear to be any more fragile than other pathogens. In fact, where comparative studies were reported *Shigella* is significantly more persistent than strains of *Salmonella* and *Vibrio cholerae* (17).

Survival studies conducted in our laboratory also indicated that *Shigella* will persist for reasonable lengths of time in aqueous systems. *Shigella flexneri* was seeded into phosphate-buffered saline (pH 6.8), sterile storm

Table 4. GENUS OF MICROORGANISMS FOUND DURING ATTEMPTS TO ISOLATE *SHIGELLA*

		Proteus sp.		*Providencia sp.*		*Pseudomonas sp.*		Others	
	Site	#/Total	% Total	#/Total	% Total	#/Total	% Total	#/Total	% Total
A.	Raw Sewage	221/720	31	173/720	24	276/720	38	50/720	7
C.	Stream	26/106	24	17/106	16	39/106	37	24/106	23
D.	Stream	81/225	36	14/225	6	41/225	18	89/225	40
	Total	328/1051	31	204/1051	19	356/1051	34	163/1051	16

Table 5. LEVELS OF INDICATOR MICROORGANISMS AND *SALMONELLA* IN SAMPLES NEGATIVE FOR *SHIGELLA*

Site	Date	TC MPN/100ml	FC MPN/100ml	FS #/100ml	*Salmonella* MPN/10 l
A. Raw Sewage	10/2 (9/30)	2.4×10^7	2.4×10^7	4.1×10^6	$>2.9 \times 10^3$
	10/7	2.4×10^7	4.9×10^6	1.1×10^6	1.2×10^3
	10/14	3.5×10^7	4.6×10^6	1.2×10^6	4.8×10^2
	10/21	2.9×10^6	4.9×10^6	8.3×10^5	5.1×10^3
	10/28	5.4×10^7	1.1×10^7	4.5×10^5	1.7×10^3
	11/4	$<2.6 \times 10^6$	$<2.6 \times 10^6$	2.0×10^4	-
	11/11	3.5×10^7	7.9×10^6	1.1×10^6	2.8×10^2
	11/18	1.7×10^7	7.9×10^6	1.1×10^6	5.1×10^2
	12/2	2.4×10^6	1.3×10^6	3.4×10^5	4.8×10^1
	12/9	7.0×10^6	3.3×10^6	9.7×10^5	1.0×10^2
	12/17	1.6×10^7	4.6×10^5	3.9×10^5	2.6×10^1
C. Stream	12/2	2.4×10^4	2.4×10^4	7.6×10^4	1.3×10^2
	12/17	9.2×10^4	3.5×10^4	4.2×10^4	2.7×10^1
D. Stream	11/4	4.6×10^2	2.3×10^2	1.2×10^2	4.4×10^0
	11/11	2.2×10^3	4.9×10^2	2.1×10^2	1.3×10^1
	11/18	1.7×10^3	3.3×10^2	1.2×10^2	2.2×10^1
	12/2	3.3×10^4	1.3×10^4	1.0×10^5	7.0×10^1
	12/9	3.5×10^4	3.3×10^3	2.4×10^4	7.0×10^1

Table 6. RECOVERY OF *SHIGELLA* ON DIATOMACEOUS EARTH PHOSPHATE-BUFFERED SALINE pH 7.2 TEMPERATURE 20-25°C

	S. sonnei		*S. flexneri*		*S. flexneri**	
	# liter	% Recovery	# liter	% Recovery	# liter	% Recovery
Shigella seeded	9	-	13	-	10	-
MPN Recovered on Celite	13	144	7	54	7	70
95% Confidence Limit for MPN	2-70	22-777	1-38	2-292	1-38	2-292

* *S. flexneri* 3 strain 3-5 Op, resistant to 500 µg/ml streptomycin

water, and stream water at a level of 3×10^6 *Shigella*/ml and stored at 40°C. Samples were removed for determination of the levels of *Shigella flexneri*. Figure 4 shows the survival of *Shigella flexneri*. After eight days 37%, 41% and 26% of the seeded organisms remained in the saline, sterile stream water, and stream water, respectively. The stream water contained 7×10^2 total coliforms, 7×10^2 fecal coliforms, 5.1×10^3 fecal streptococci, 3.3×10^2 *Pseudomonas aeruginosa* and less than 1.8 *Staphlococcus aureus* per 100 ml and 2.7×10^1 *Salmonella*/10 liters. The stream water pH was 7.5.

The major difficulty at present appears to be the enrichment step. Enrichment is necessary because of the low levels of *Shigella* present in the aquatic environment. The antagonism between the normal intestinal flora and enteric pathogens has received attention for quite some time. Hentges (15) recently reported the ability of 22 strains of microorganisms to suppress the growth of *Shigella* in the mouse intestine. All strains of *Escherichia coli* and *Enterobacter aerogenes* and most strains of *Proteus vlugaris* tested were antagonistic to *Shigella*. The bacteriostatic and bactericidal effects of the coliform organisms were attributed to volatile acids produced as metabolic end products. The effects were enhanced at lower pH values.

The application of the findings of Hentges (15) to the development of a suitable enrichment procedure has yielded some success. Enrichment conditions were set up to minimize the production and effect of volatile acids. Nutrient broth, a complex medium which contains low levels of carbohydrates, was adjusted to pH 8.0 and inocualted with 59 *Shigella sonnei* and *Shigella flexneri*. One ml of stream water or 1 ml of raw sewage was added as a source of interfering microorganisms. comparable cultures were set up with GN broth. Replicate cultures were incubated under aerated and stationary conditions. *Shigella sonnei* was recovered from the flasks seeded with stream water with an initial pH of 8.0. The predominant interference was members of the genus *Pseudomonas*. One subsequent attempt to recover low levels of *Shigella* (14 *S. sonnei*, 28 *S. flexneri* and 9 *S. flexneri*) did not produce any *Shigella* isolates. Of 148 suspicious colonies, 129 were tentatively identified as *Pseudomonas*. The minimal content of carbohydrate in the medium and the aerated conditions favored the growth of the *Pseudomonas*. Experiments conducted in the laboratory indicate that the growth of laboratory cultures of *Pseudomonas aeruginosa* was not antagonistic to *Shigella* and the results are in agreement with the reports of Hentges (15). *Pseudomonas* apparently masks the presence of *Shigella*.

STAPHYLOCOCCUS aureus

Serious difficulties were encountered with the determination of levels of *Staphylococcus aureus*. Membrane filter procedures using m-staphylococcus broth and Vogel-Johnson medium (V-J) and plate counts on tellurite glycine agar were evaluated with samples of sewage and urban streams. Each medium yielded many suspicious *Staph. aureus* colonies with typical morphology. However, those colonies failed to yield typical biochemical reactions, Gram stains and microscopic morphology. Table 7 shows the recovery of confirmed *Staph. aureus* for 1,249 isolates from four procedures for 66 samples of raw sewage and urban streams. High levels of interfering microorganisms were found with m-staphylococcus broth, tellurite glycine and VJ medium. The

Figure 4. Survival of *Shigella flexneri* at 4°C

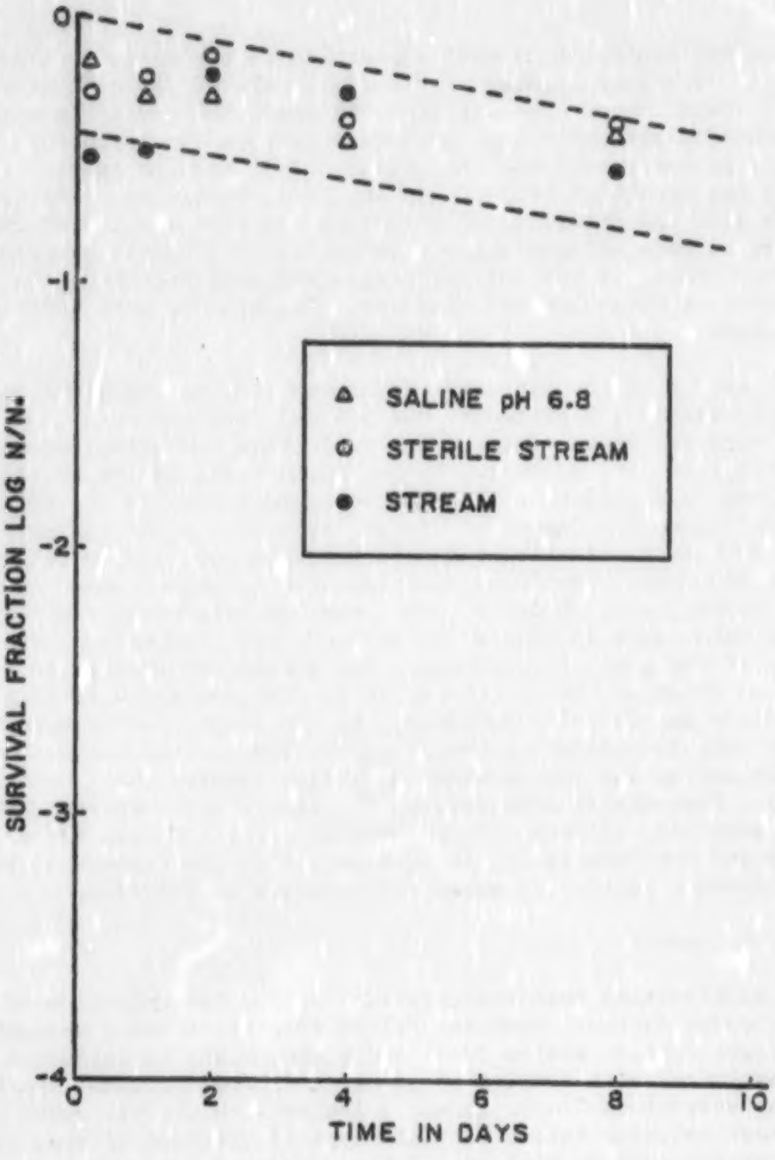


Table 7. RECOVERY OF *STAPH. AUREUS*

Medium	Procedure	Number of samples	Number of typical isolates	Number of confirmed *Staph. aureus*	% confirmed *Staph. aureus*
Tellurite-glycine	plate count	19	265	7	2.6
Vogel-Johnson	membrane filter	13	109	1	0.9
M-staphylococcus broth	membrane filter	16	344	30	8.7
M-staphylococcus broth + 0.75mM azide enrichment and LSM primary plate	MPN	18	531	507	95.4
Totals		66	1,249	-	-

predominant interference on tellurite glycine was Gram positive cocci that were catalase negative, DNase negative and yielded pink to red colonies of KF streptococcus agar. The typical colony morphology expected for *Staph. aureus* on V-J medium is a black colony surrounded by a yellow halo which indicates mannitol fermentation. Stock *Staph. aureus* on V-J medium is a black colony surrounded by a yellow halo which indicates mannitol fermentation. Stock *Staph. aureus* cultures when collected on membrane filters and grown on V-J medium did yield black colonies but the yellow halo was obscured by the membrane filter and difficult to detect. The isolates obtained from the membrane filters incubated on m-staphylococcus broth were predominantly Gram positive and Gram negative, rod-shaped bacteria. In each case the presence of *Staph. aureus* was heavily masked by interfering microorganisms that gave typical colony morphology. Simple enumeration of suspicious colonies would yield a gross overestimate of the levels of *Staph. aureus* for each sample.

Smuckler and Appleman (21) reported similar difficulties with staphylococcus medium 110 when attempting to enumerate *Staph. aureus* in meat pot pies. Staphylococcus medium 110 was the medium from which m-staphylococcus broth was developed for use with membrane filters. The former was prepared as a solid medium with agar and contains gelatin. The latter is a broth without gelatin. Similar to our observations with m-staphylococcus broth, Smuckler and Appleman (21) observed high levels of interfering rod-shaped bacteria that yield typical colonies on staphylococcus medium 110. After testing several inhibitors they recommended the addition of 0.75 mM sodium azide to the staphylococcus medium 110 to inhibit the growth of the rod-shaped bacteria.

Sodium azide was added to m-staphylococcus broth at a concentration of 0.75 mM and the modified medium was employed as an enrichment broth for *Staph. aureus*. A multiple tube dilution procedure was utilized to permit the calculation of a MPN. Lipovitellenin-salt-mannitol agar (LSM) was employed as a primary isolation medium from the modified m-staphylococcus broth. Gunn *et al.* (10) used LSM to isolate and identify *Staph. aureus*. The production of opaque yellow zones around the colonies was considered positive evidence for lipovitellenin-lipase activity (opaque) and mannitol fermentation (yellow). Lipovitellenin-lipase activity was found to correlate with coagulase production for 94.7% of the isolates tested by Gunn *et al.* (10). Table 7 shows the recovery of *Staph. aureus* for typical colonies on LSM after enrichment in the modified m-staphyloccus broth with 0.75 mM sodium azide. 507 of 531 or 95.4% of the typical isolates after enrichment and plating on LSM were confirmed *Staph. aureus*.

The correlation of biochemical characteristics with pathogenicity of staphylococcus isolates has been the subject of numerous reports. Unfortunately, no one criterion is adequate for the identification of *Staph. aureus*. Even coagulase production, which has traditionally been used to differentiate *Staph. aureus* from *Staph. epidermis* (formerly *Staph. albus*) is not absolute (22). The enumeration must be based on several biochemical characteristics that will enable a differentiation between not only *Staph. epidermis* but other genera of microorganisms not normally encountered at high levels in clinical samples.

Enrichment tubes in the multiple dilutions procedure were considered positive when isolates were recovered that were catalase negative, coagulase positive, DNase positive, fermented mannitol, fermented glucose anaerobically, yielded typical microscopic morphology and Gram positive. A schematic of the culture procedure is given in Figure 5.

PSEUDOMONAS aeruginosa

Culture media for the enumeration of *Pseudomonas aeruginosa* were evaluated with samples of raw sewage and urban streams. MPN procedures using L-asparagine broth and confirmation on acetamide broth according to *Standard Methods* (5), Drake's asparagine broth #10 (23) confirmed on acetamide broth and membrane filter procedures using MacConkey agar according to Nixon and Brodsky (24) were tested. Table 8 shows the results of the initial culture evaluation for 16 samples of raw sewage and urban streams. L-asparagine broth according to *Standard Methods* yielded consistently higher levels of confirmed *Pseudomonas aeruginosa* than either of the other methods tested and was employed in the remainder of the study. Spot inoculation on acetamide agar (acetamide broth + 1.5% agar) was compared to acetamide broth for a confirmatory procedure to permit the use of acetamide agar as a one step isolation and confirmatory procedure and as confirmatory screening procedure before streak isolation. Table 9 shows the comparative levels of *P. aeruginosa* calculated from confirmation on acetamide broth and acetamide agar. Each presumptive positive dilution tube was transferred for confirmation to acetamide broth and spotted or streaked on acetamide agar. The levels of *P. aeruginosa* were similar for each confirmatory method except for one stream sample.

The final procedure for the enumeration and identification of *P. aeruginosa* is given in schematic form in Figure 6. Asparagine tubes showing growth with fluorescence after incubation at 37°C for 48 hours were streaked on acetamide agar or spotted on acetamide agar and subsequently streaked on PAP agar for isolation. Isolates were submitted to the tests indicated for verification of *P. aeruginosa*. Calculation of the MPN *P. aeruginosa* was based on the recovery of isolates that were acetamide and oxidase positive and grew at 42°C. Casein hydrolysis with fluorescence (25) was employed as a secondary characteristic.

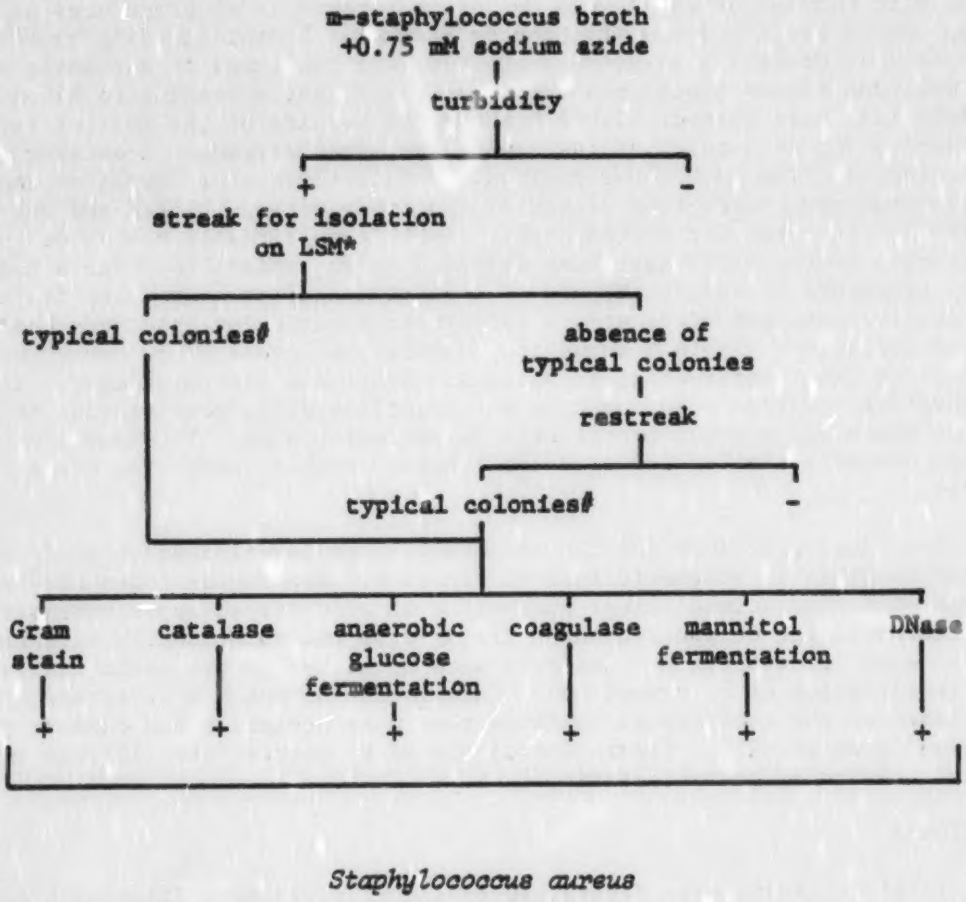
COLIFORMS

Total coliforms were determined by the multiple tube dilution procedure with lactose broth as the presumptive medium according to *Standard Methods* (5). Positive tubes were confirmed on brilliant green lactose broth with 2% bile.

Fecal coliforms were determined by confirmation of positive lactose broth presumptive tubes on EC medium incubated at 44.5°C for 24 hours (5).

The effect of homogenization on the levels of microorganisms was evaluated. Stream samples were blended at room temperature for varying time periods and the levels of total and fecal coliforms determined. The results

Figure 5. Schematic - Isolation and identification of *Staphylococcus aureus*


m-staphylococcus broth
+0.75 mM sodium azide
turbidity
+
-
streak for isolation
on LSM*
typical colonies#
absence of
typical colonies
restreak
typical colonies#
-
Gram
stain
catalase
anaerobic
glucose
fermentation
coagulase
mannitol
fermentation
DNase
+
+
+
+
+
+
Staphylococcus aureus

* LSM - lipovitellenin salt mannitol agar

# typical colonies - colonies surrounded by opaque and/or yellow zones

Table 8. EVALUATION OF PRESUMPTIVE MEDIA FOR THE ENUMERATION OF *PSEUDOMONAS AERUGINOSA*

	L-Asparagine broth a MPN/100ml	Drakes #10 broth a MPN/100ml	MacConkey agar - mf a b #/100ml
Raw Sewage	9.2×10^5	3.5×10^5	4.5×10^5
	3.5×10^5	2.3×10^5	ND
	2.3×10^6	9.2×10^5	4.5×10^6
	1.7×10^6	4.6×10^5	1.0×10^6
Stream	1.7×10^2	1.1×10^2	1.4×10^2
	1.7×10^3	1.4×10^2	4.0×10^3
	1.6×10^5	1.6×10^5	2.0×10^4
	2.2×10^3	1.1×10^2	ND
	2.3×10^4	2.2×10^4	1.1×10^3
	2.4×10^4	1.7×10^3	3.0×10^4
	3.5×10^5	1.9×10^4	2.0×10^4
	1.1×10^3	3.1×10^2	4.0×10^3
	1.7×10^5	3.3×10^4	1.5×10^4
	2.8×10^4	4.9×10^3	1.5×10^4
	7.0×10^4	3.3×10^3	1.0×10^4
	5.4×10^3	7.9×10^2	1.5×10^3

a Confirmed in acetamide broth

ND No data

b Brodsky and Nixon (24)

Table 9. LEVELS OF *PSEUDOMONAS AERUGINOSA* CONFIRMED ON ACETAMIDE BROTH AND ACETAMIDE AGAR

	Pseudomonas aeruginosa MPN/100ml Acetamide confirmation	
Sample	Broth	Agar
Raw Sewage	2.3×10^6	2.3×10^6
	1.7×10^6	2.8×10^6
	9.2×10^5	1.6×10^6
	4.6×10^5	1.7×10^5
Stream	1.6×10^5	2.2×10^4
	2.4×10^4	2.4×10^4
	2.8×10^4	7.0×10^4
	2.2×10^3	3.5×10^3
	3.5×10^5	1.6×10^5
	1.6×10^5	1.6×10^5
	1.7×10^3	1.3×10^3
	4.9×10^3	1.3×10^4
	1.7×10^4	1.8×10^3
	3.3×10^3	3.5×10^3

Figure 6. Schematic - Identification of *Pseudomonas aeruginosa*

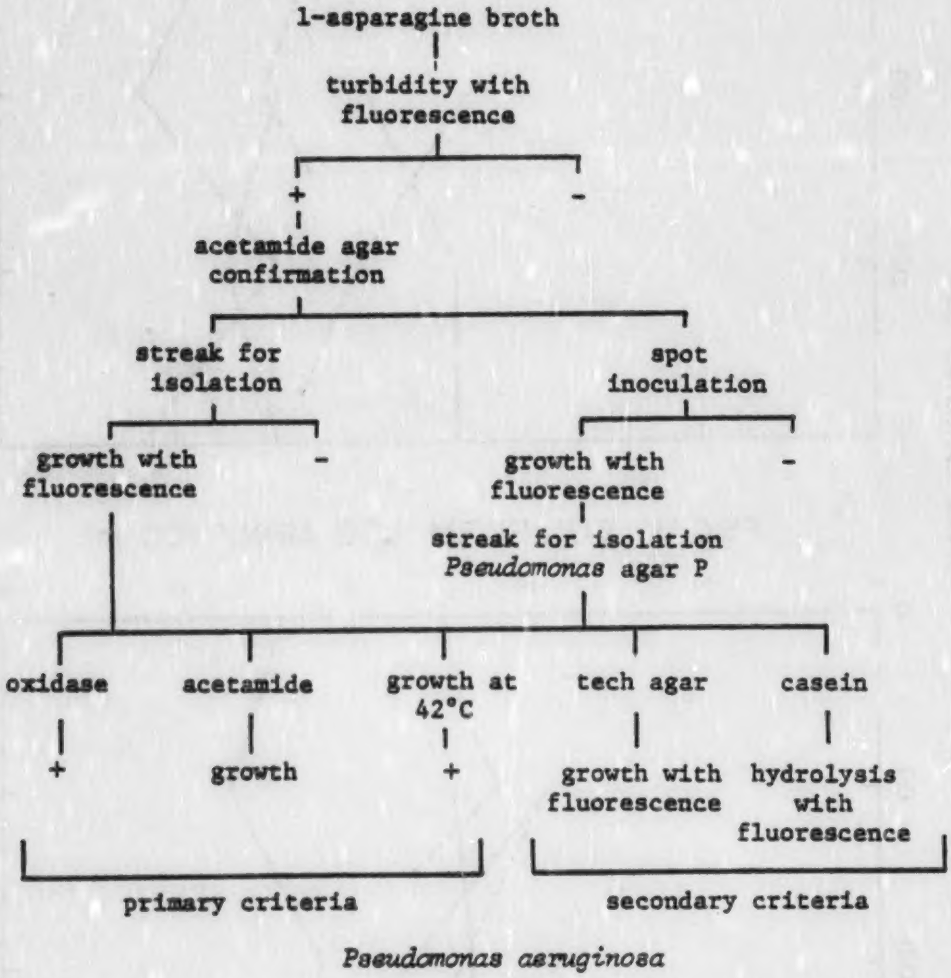

l-asparagine broth
turbidity with fluorescence
+
-
acetamide agar confirmation
streak for isolation
spot inoculation
growth with fluorescence
-
growth with fluorescence
-
streak for isolation *Pseudomonas* agar P
oxidase
acetamide
growth at 42°C
tech agar
casein
+
growth
+
growth with fluorescence
hydrolysis with fluorescence
primary criteria
secondary criteria
Pseudomonas aeruginosa

Figure 7. Effect of homogenization on the levels of total and fecal coliforms

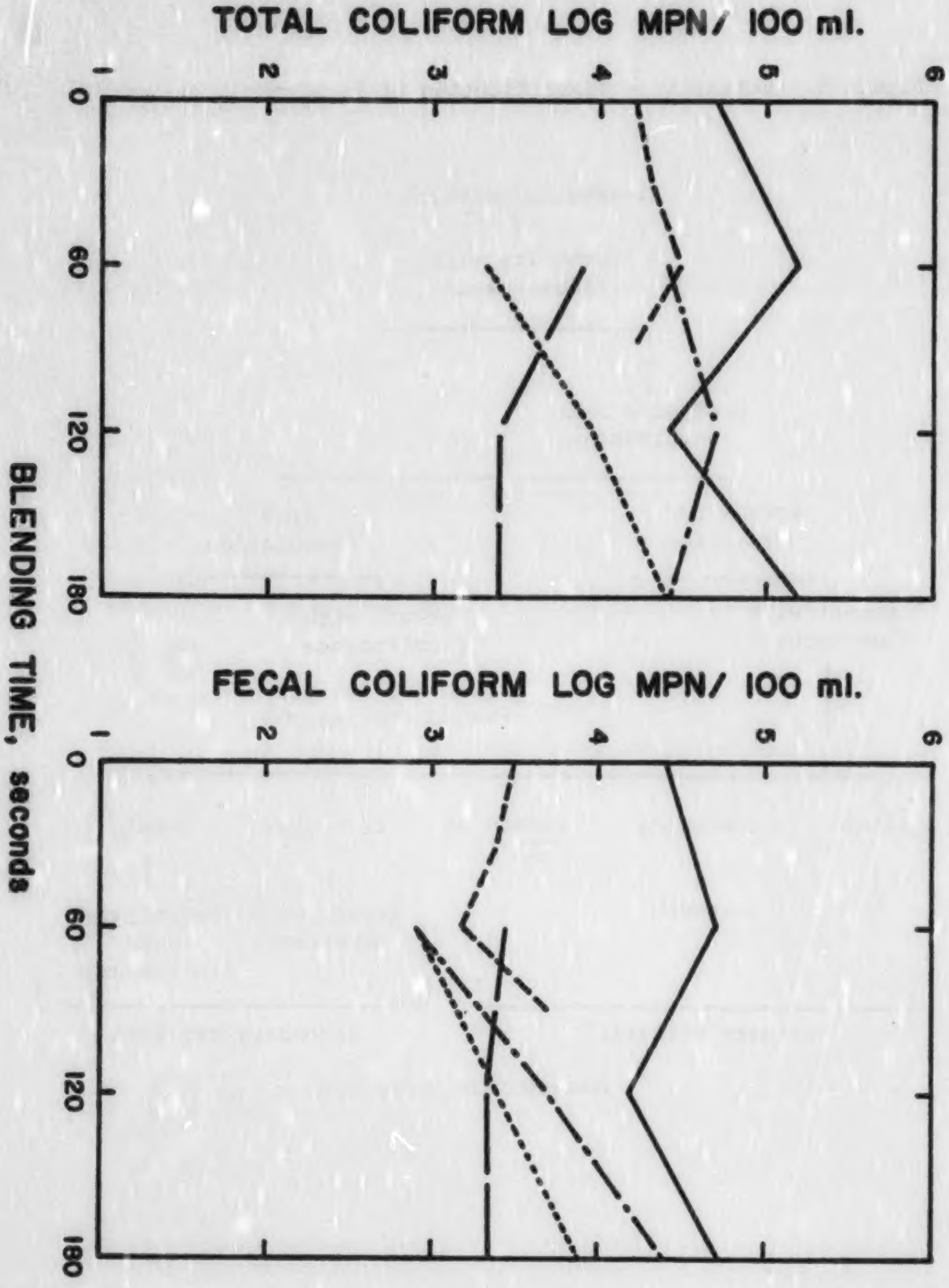
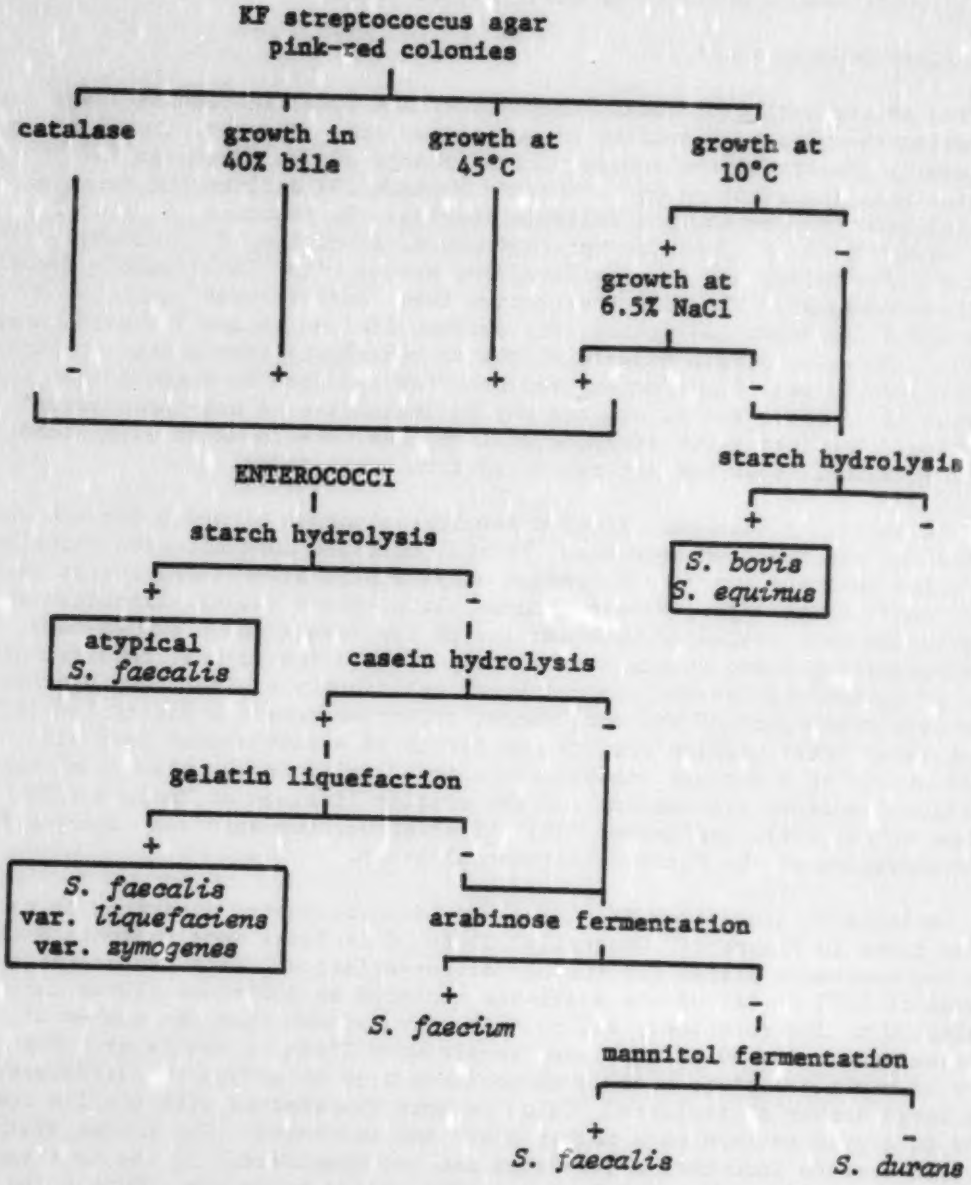


Figure 8. Identification of the fecal streptococci


KF streptococcus agar
pink-red colonies
catalase
growth in 40% bile
growth at 45°C
growth at 10°C
+
-
growth at 6.5% NaCl
+
-
-
+
+
+
-
ENTEROCOCCI
starch hydrolysis
+
-
atypical S. faecalis
casein hydrolysis
+
-
gelatin liquefaction
+
-
S. faecalis var. liquefaciens var. zymogenes
arabinose fermentation
+
-
S. faecium
mannitol fermentation
+
-
S. faecalis
S. durans
starch hydrolysis
+
-
S. bovis
S. equinus

for five trials are shown in Figure 7. No consistent trends and little significant differences were observed for the levels of total coliforms after blending for time periods up to 180 seconds. Two trials showed an increase in levels of fecal coliform while three trials showed little significant differences with blending time. Again no consistent trends were observed and no blending time necessary for optimum levels of coliforms could be predicted. As a result, a preparative blending step for each sample before the microbial assays would be of questionable value.

FECAL STREPTOCOCCAL GROUP

The determination of fecal streptococci has found increasing usage for evaluating the microbial quality of surface waters. However, there is some controversy concerning the sanitary significance of the microorganisms that comprise this indicator group. *Standard Methods* (5) defines the fecal streptococcal group to contain the followin species: *S. faecalis*, *S. faecalis* var. *liquifaciens*, *S. faecalis* var. *zymogenes*, *S. durans*, *S. faecium*, *S. bovis* and *S. equinus* and is considered synonymous with "Lancefield's Group D. Streptococcus". The more restrictive term "enterococcus" excludes *S. bovis* and *S. equinus*. Geldreich (26) assigns limited sanitary significance to the *S. faecalis* strain capable of starch hydrolysis (designated atypical) and *S. faecalis* var. *liquifaciens* and provides evidence to suggest that the subgroup of *S. bovis* and *S. equinus* may be indicative of non-human animal pollution. The latter two streptococcal species were found in significant levels of animal feces but not recovered from human feces.

The initial effort was directed toward evaluation of media for the enumeration of the fecal streptococcal group. Multiple tube dilution technique with azide dextrose broth confirmed on ethyl violet azide (EVA) and/or enterococci confirmatory agar, M-enterococcus plate counts and KF streptococcus plate counts were evaluated with samples of raw sewage and urban streams. KF streptococcus plate counts consistently yielded the highest recovery of fecal streptococci. M-enterococcus agar consistently yielded low recovery. Subsequent evaluation of the more recent selective enterococcus medium (Pfizer Diagnostics) (PSE) yielded results similar to KF streptococcus agar with the advantage of a shorter incubation period. Based on the results of the preliminary culture evaluations and the similar findings of Pavlova (27), Hartman *et al.* (28), and Kenner (29), KF streptococcus agar was employed for the enumeration of the fecal streptococcal group.

Isolates obtained from KF plates were differentiated according to the scheme given in Figure 8. Generally, 35 to 50 isolates were randomly picked from the countable plates for further differentiation. This represents a minimum of 5.7% to 58% of the available isolates on duplicate plates containing 30 to 300 colonies. All colonies were picked when the number of colonies were below 30 at the lower sensitivity limit of the assay. Two types of replicate plate procedures were employed to enable the differentiation of a large number of isolates. Colonies were transferred with sterile toothpicks to a grid pattern on a master plate and incubated. The master plate was then used to inoculate a velveteen pad and transferred in the grid pattern to the appropriate differential agars. The second procedure employed the preparation of a master plate as above but a multiple point inoculation device utilizing toothpicks was used. Each isolate was picked from the master plate with a sterile toothpick and transferred to a plexiglass toothpick holder in a similar grid pattern. The charged toothpicks were then used to inoculate the appropriate differential agars. The last plate in each replication series was a BHI agar control to evaluate the transfer. Isolates not replicated through the final control plate were not considered. Each procedure would easily transfer microorganisms through 15 plates.

The velveteen and toothpick replicator were evaluated by comparing results obtained in simultaneous determinations with conventional tube methods. The results for 250 trials of isolates obtained from KF streptococcus agar is given in Table 10. With the exception of arabinose fermentation 94.4% agreement or better was observed between the replication procedures and standard tube methods. The relatively poor agreement on arabinose fermentation appears to be a function of the initial pH of the medium. If the initial pH of the medium is too low the fermentation reaction was difficult to read. Subsequent comparison with 50 isolates where the pH was adjusted to 7.4 before autoclaving yielded 98% agreement. The overall agreement on species identification by the replication procedures and the tube methods was 93.0% for the velveteen technique and 94.5% for the toothpick method. Many of the isolates that did not agree with the tube procedures for arabinose fermentation did not require arabinose fermentation for the determination of species. Considering the group identification of enterococcus, *S. bovis* and *equinus*, the *liquefaciens* and *zymogenes* variations of *S. faecalis*, atypical *S. faecalis* and false positives, the percent agreement for the overall group identification was 97.0% for both procedures.

Both replication procedures were rapid, inexpensive and reasonably accurate. The toothpick replicator yielded a more positive (small noticeable holes in the agar at the time of replication) inoculation of the agar and was less sensitive to plate moisture than the velveteen replication technique. The major portion of the assays was conducted with the toothpick replicator.

Table 10. COMPARISON OF VELVETEEN AND TOOTHPICK REPLICATION PROCEDURES TO CONVENTIONAL TUBE METHODS FOR THE DIFFERENTIATION OF FECAL STREPTOCOCCI

Test	# of tests	Velveteen # agree	Velveteen % agreement	Toothpick # agree	Toothpick % agreement
Growth in 40% bile	250	250	100	250	100
Growth in 6.5% NaCl	250	237	94.8	236	94.4
Growth at 45°C	250	242	96.8	243	97.2
Growth at 10°C	250	242	96.8	240	96.0
Starch hydrolysis	250	-	-	-	-
Gelatin liquafaction	150	150	100	150	100
Casein hydrolysis [a]	250	250	100	250	100
Arabinose fermentation	200	166	83.0	184	92.0
Mannitol fermentation	250	240	96.0	243	97.2
Species identification [b]	-	-	93.0	-	94.5
Group identification	-	-	97.0	-	97.0

[a] Casein hydrolysis was compared to peptonization of litmus milk

[b] *S. durans* classified as a variation of *S. faecium*

RESULTS

A comprehensive presentation and discussion of the information obtained during the current investigation is beyond the scope of this report. Some highlights and observed trends are given below.

The ranges in the levels of microorganisms found in the background and stormwater samples are given in Table 11 and 12, respectively. The minimum and maximum levels of indicator and pathogenic microorganisms observed are reported as MPN or number per 100 ml with the exception of the data for *Salmonella* sp. which is reported as MPN *Salmonella*/10 liters. The final two columns are the minimum and maximum levels for the ratio of fecal coliform to total coliform and fecal coliform to fecal streptococci. It is evident from both tables that the ranges of the levels of the different microorganisms and the magnitude of the ratios of indicator microorganisms varies widely for each sample station. Differences between the minimum and maximum levels of several orders of magnitude are observed in each case for each microorganism group. The wide variations are not unexpected. Each sample is a grab sample and reflects the microbial quality at the time the sample was taken. Large differences in flow, quantity of rainfall, antecedent rainfall, and portion of the stormwater sampled (first flush, mid-storm, tail) were observed from sample to sample at each station. These wide variations emphasize the need to obtain a large number of samples over a reasonable length of time to provide valid information to evaluate trends and to observe any specific correlation between the levels of different microorganisms.

Table 13 shows the frequency of detection of *Salmonella* at different levels of fecal coliforms and a comparison of similar data reported in the literature. The levels of fecal coliforms in raw sewage exceeded 2,000 per 100 ml in each sample, and *Salmonella* was isolated in every case. The samples collected and assayed from an upland reservoir contained low levels of fecal coliforms. *Salmonella* was isolated from one sample. The majority of the samples collected from urban streams and stormwater contained greater than 2,000 fecal coliforms per 100 ml. *Salmonella* was found routinely. The exception was not isolating *Salmonella* from samples of urban stremas and stormwater. For the overall total of 273 samples collected from the urban aquatic environment, *Salmonella* was isolated in 27%, 89% and 96% of the samples containing 0-200, 201-2,000 and greater than 2,000 fecal coliforms per 100 ml, respectively. The frequency of *Salmonella* isolation compares favorably with the fresh water data reported by Geldreich and Van Donsel (30) and differs significantly from the estuary data reported by these authors and Brezenski and Russomanno (31).

An important aspect of the current study has been in inclusion of seeded *Salmonella* controls to evaluate the steps in the recovery of *Salmonella*. Table 14 shows the frequency of recovery of the seeded *Salmonella* for each sample station. Only two storm samples were found to cause more than 90% inactivation of the seeded *Salmonella*. The test organism was consistently recovered after exposure to the water samples from the different sources and indicated that the water samples were not bactericidal to the seeded *Salmonella*. After concentration on diatomaceous earth, the seed *Salmonella* was recovered in 67% to 100% of the samples depending on sample

Table 11. LEVELS OF MICROORGANISMS IN RAW SEWAGE AND URBAN STREAMS

Station	Number of Samples	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml	Fecal Streptococci MPN/100ml	*Pseudomonas aeruginosa* MPN/100ml	*Staph. aureus* MPN/100ml	*Salmonella* sp. MPN/10 liters	FC/TC	FC/FS
Raw Sewage	34								
min.		1.3×10^6	3.3×10^5	2.0×10^4	3.3×10^3	4.3×10^1	2.6×10^1	.03	1.2
max.		1.6×10^9	9.2×10^8	3.3×10^6	5.4×10^7	4.6×10^3	2.7×10^4	1.0	25.0
Herring Run	34								
min.		5.0×10^1	2.0×10^1	1.9×10^2	5.0×10^0	$<2.0 \times 10^0$	$<9.0 \times 10^1$	.08	< .1
max.		3.5×10^5	3.5×10^5	4.0×10^4	1.6×10^5	7.0×10^0	1.3×10^2	1.0	21.9
Jones Falls	34								
min.		1.1×10^4	1.1×10^4	2.6×10^3	1.1×10^2	2.0×10^0	1.0×10^0	.15	.3
max.		3.3×10^5	2.4×10^5	9.2×10^4	3.5×10^5	1.8×10^2	3.2×10^2	1.0	46.9
Gwynns Falls	34								
min.		3.4×10^2	8.0×10^1	$<1.0 \times 10^2$	3.0×10^0	$<1.0 \times 10^0$	4.1×10^0	.03	.13
max.		2.4×10^6	2.4×10^6	$>1.0 \times 10^5$	1.7×10^5	9.3×10^1	1.3×10^2	1.0	37.7
Loch Raven	8								
min.		$<2.0 \times 10^0$	$<2.0 \times 10^0$	$<5.0 \times 10^0$	$<2.0 \times 10^0$	$<3.0 \times 10^0$	$<8.8 \times 10^{-1}$	0.5	.2
max.		4.0×10^2	8.0×10^1	2.0×10^2	2.3×10^1		8.8×10^{-1}	1.0	.4
	144								

Table 12. LEVELS OF MICROORGANISMS IN STORMWATER RUNOFF

Station	Number of Samples	Total Coliform MPN/100ml	Fecal Coliform MPN/100ml	Fecal Streptococci MPN/100ml	*Pseudomonas aeruginosa* MPN/100ml	*Staph. aureus* MPN/100ml	*Salmonella* sp. MPN/10 liters	FC/TC	FC/FS
Stoney Run	17								
min.		5.4×10^3	1.3×10^3	5.3×10^2	2.3×10^2	$<3.0 \times 10^0$	2.9×10^0	< .07	.06
max.		1.6×10^6	5.4×10^4	3.0×10^5	2.4×10^5	7.9×10^1	$>1.3 \times 10^3$	1.0	92.5
Glen Ave.	17								
min.		7.9×10^3	1.4×10^3	9.2×10^3	1.3×10^2	$<3.0 \times 10^0$	1.7×10^0	.06	.02
max.		1.6×10^6	2.3×10^5	2.8×10^6	2.6×10^5	1.5×10^2	$>1.1 \times 10^4$	1.0	5.9
Howard Park	17								
min.		4.9×10^3	2.3×10^3	$<1.0 \times 10^3$	7.9×10^2	6.0×10^0	3.9×10^0	.02	.05
max.		2.8×10^7	2.9×10^6	1.4×10^6	5.4×10^4	9.2×10^2	$>1.3 \times 10^3$	1.0	32.7
Jones Falls	17								
min.		3.3×10^4	5.0×10^3	2.6×10^3	9.4×10^2	4.0×10^0	1.7×10	.01	.06
max.		$>2.4 \times 10^6$	$>1.6 \times 10^6$	8.0×10^5	1.6×10^6	1.1×10^1	2.7×10	1.0	4.4
Bush Street	17								
min.		7.9×10^3	1.7×10^3	2.5×10^3	1.1×10^2	$<3.0 \times 10^0$	$<1.7 \times 10^0$	.05	.02
max.		2.4×10^6	2.4×10^6	1.9×10^6	7.5×10^4	4.6×10^3	2.7×10^3	1.0	12.9
Northwood	14								
min.		1.3×10^3	8.0×10^1	1.7×10^3	1.7×10^1	$<3.0 \times 10^0$	$<1.7 \times 10^0$	.01	.01
max.		1.7×10^5	7.9×10^4	3.0×10^5	9.2×10^3	4.6×10^2	4.3×10^1	.65	4.6
	99								

Table 13. COMPARISON OF THE FREQUENCY OF DETECTION OF *SALMONELLA* WITH THE LEVELS OF FECAL COLIFORMS

		Fecal Coliform		*Salmonella*	
Report	Sample	Range MPN/100ml	Number of Samples in Range	Number of Samples Positive	Percent Positive
J.H.U.	raw sewage	0-200	0		
		201-2000	0		
		>2000	32	32	100
	urban streams	0-200	3	3	100
		201-2000	34	31	91
		>2000	55	53	96
	upland reservoir	0-200	13	1	8
		201-2000	0		
		>2000	0		
	storm runoff	0-200	1	1	100
		201-2000	12	10	83
		>2000	123	117	95
	overall total	0-200	17	5	29
		201-2000	46	41	89
		>2000	210	202	96
Geldreich & Van Donsel (30)	fresh water	0-200	29	19	27
		201-2000	27	53	70
		>2000	54	33	98
	estuary	0-200	258	33	13
		201-2000	91	40	44
		>2000	75	45	60
Brezenski & Russomanno (31)	estuary	0-200	34	6	18
		201-2000	43	13	30
		>2000	73	43	59

Table 14. FREQUENCY OF RECOVERY OF SEEDED *SALMONELLA* AFTER EXPOSURE TO THE SAMPLE, CONCENTRATION ON DIATOMACEOUS EARTH AND ENRICHMENT WITH PRIMARY PLATING

Sample station	% of the samples positive Exposure [a]	Concentration	Enrichment with primary plating
Background			
Raw sewage	100	100	0
Herring Run	100	88	67
Jones Falls	100	88	17
Gwynns Falls	100	82	33
Loch Raven	100	100	83
Storm drain			
Stoney Run	100	79	0
Glen Ave.	100	93	25
Howard Park	95	100	0
Jones Falls	95	79	0
Bush Street	100	86	50
Northwood	100	67	25
All samples	99	87	30

[a] Recovery of the seeded *Salmonella* after exposure to the sample was considered positive if less than 90% inactivation was observed.

station. The *Salmonella* seed was recovered in 87% of all samples. This suggests that the diatomaceous earth concentration procedure for the recovery of low levels of *Salmonella* from large volumes of water was effective. After enrichment and primary plating, the recovery of the seeded *Salmonella* decreases markedly and was recovered in only 30% of the samples. The frequency of recovery for the overall culture procedure varied with the sample site. In general, the samples with the higher levels of microorganisms yielded poorer recoveries, and samples with low levels of microorganisms yielded better recoveries. The data suggest that the major difficulty with the procedures for the detection and enumeration of *Salmonella* in water lies in the enrichment step.

The relationship between the logarithm of the levels of *Salmonella* sp. (MPN/10 liters) and the logarithm of the levels of total coliform (MPN/100 ml), fecal coliform (MPN/100 ml) and fecal streptococci (#/100 ml) can be seen in Figures 10, 11, and 12, respectively. In each case there is some relationship between the pathogen and the indicator group. However, there is a noticable difference between the least squares curve for the background samples and the storm water samples. Table 15 summarizes the correlation coefficients (r) for the levels of *Salmonella* and indicator microorganisms. Good correlation (r = ~ 0.7) was observed for total coliform, fecal coliform, fecal streptococci and enterococci for the background samples. For the stormwater samples the correlation coefficient was far less significant particularly for the fecal streptococci and enterococci. The correlation coefficient between indicator microorganism and *Salmonella* when all samples are considered was between 0.53 and 0.59. The data suggest that the levels of indicator microorganisms are less meaningful in stormwater runoff.

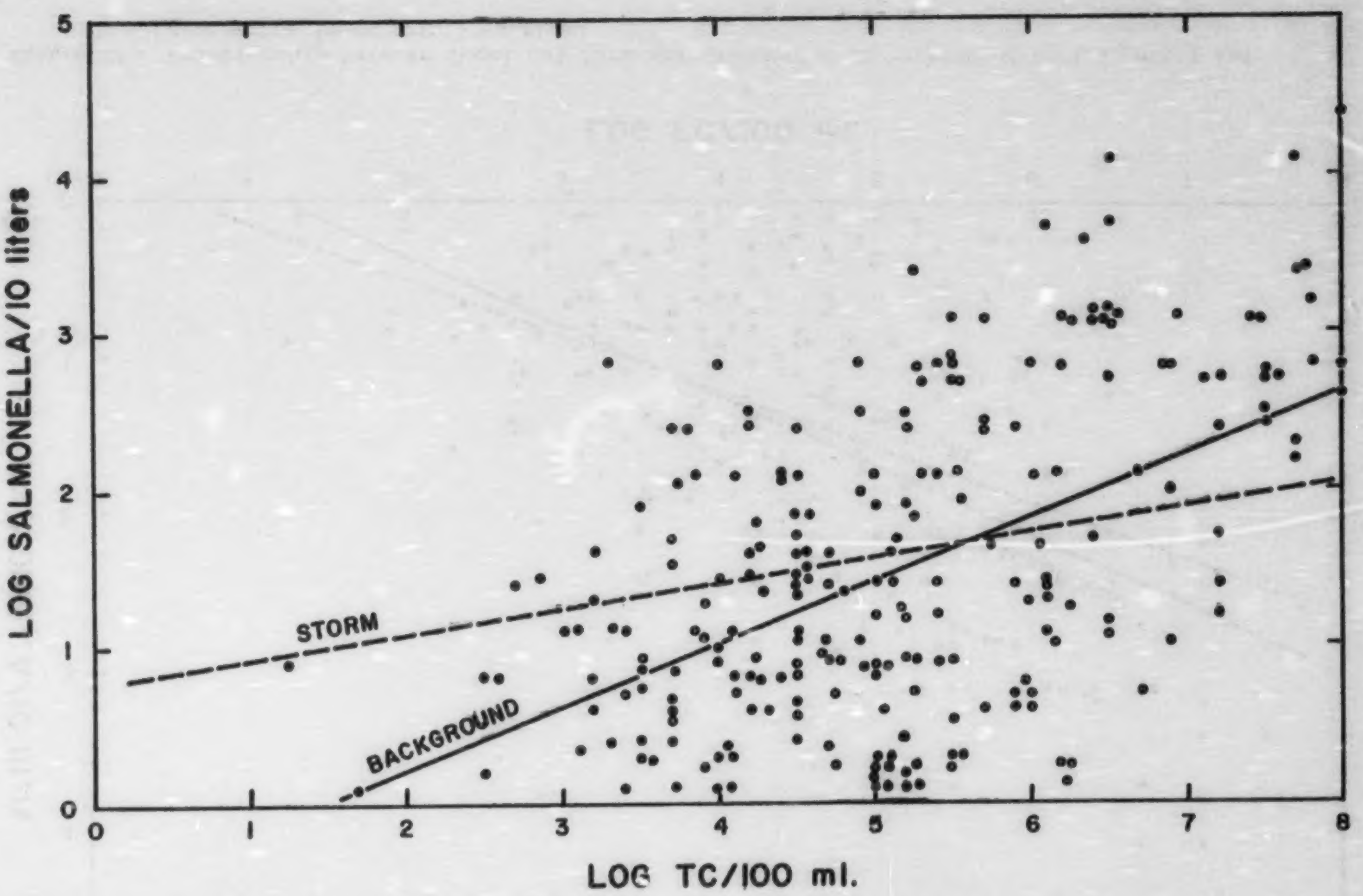


Figure 10. Relationship between total coliform and *Salmonella* in background (solid point) and stormwater (open point) samples.

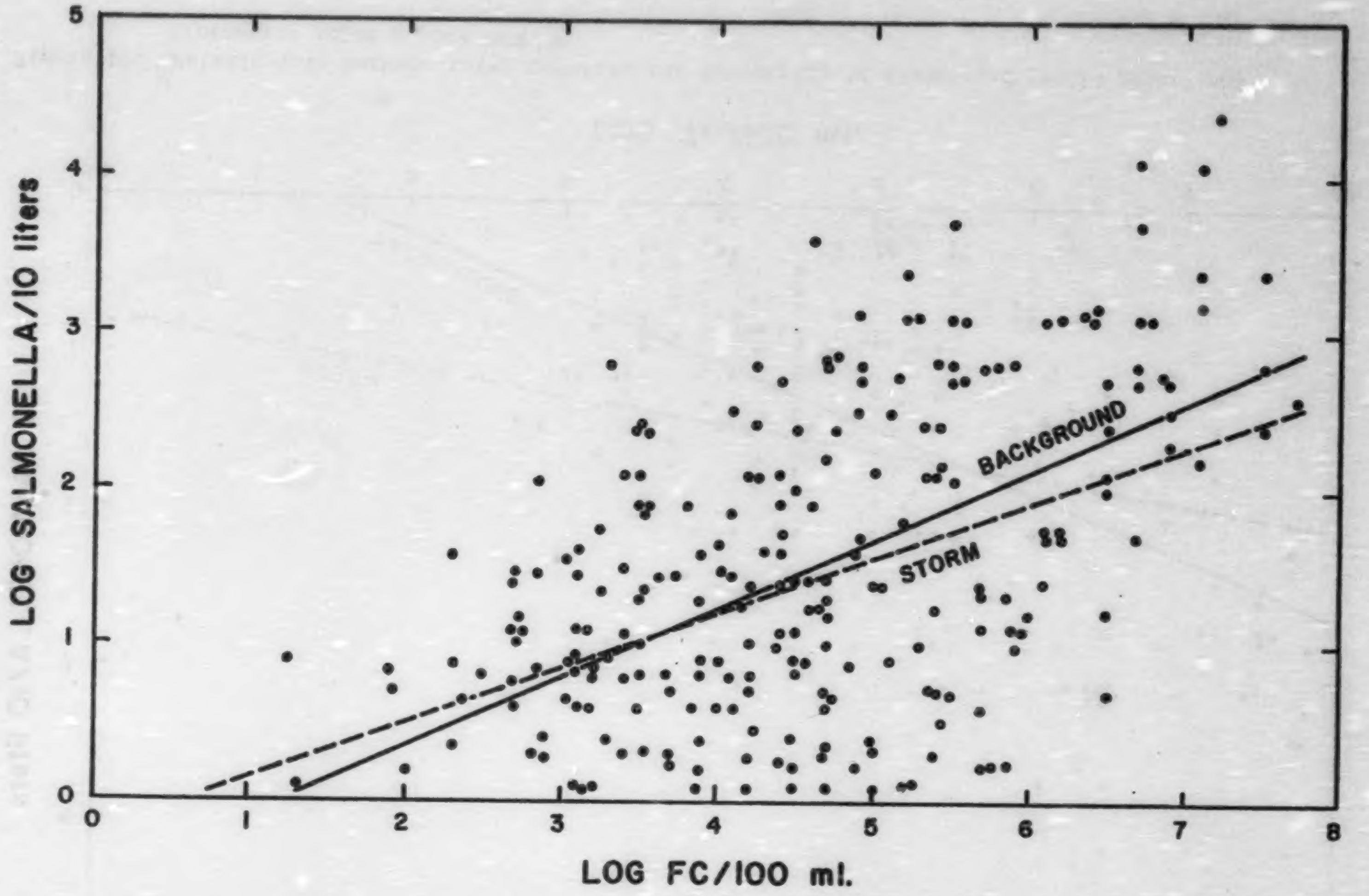


Figure 11. Relationship between fecal coliform and *Salmonella* in background (solid point) and stormwater (open point) samples.

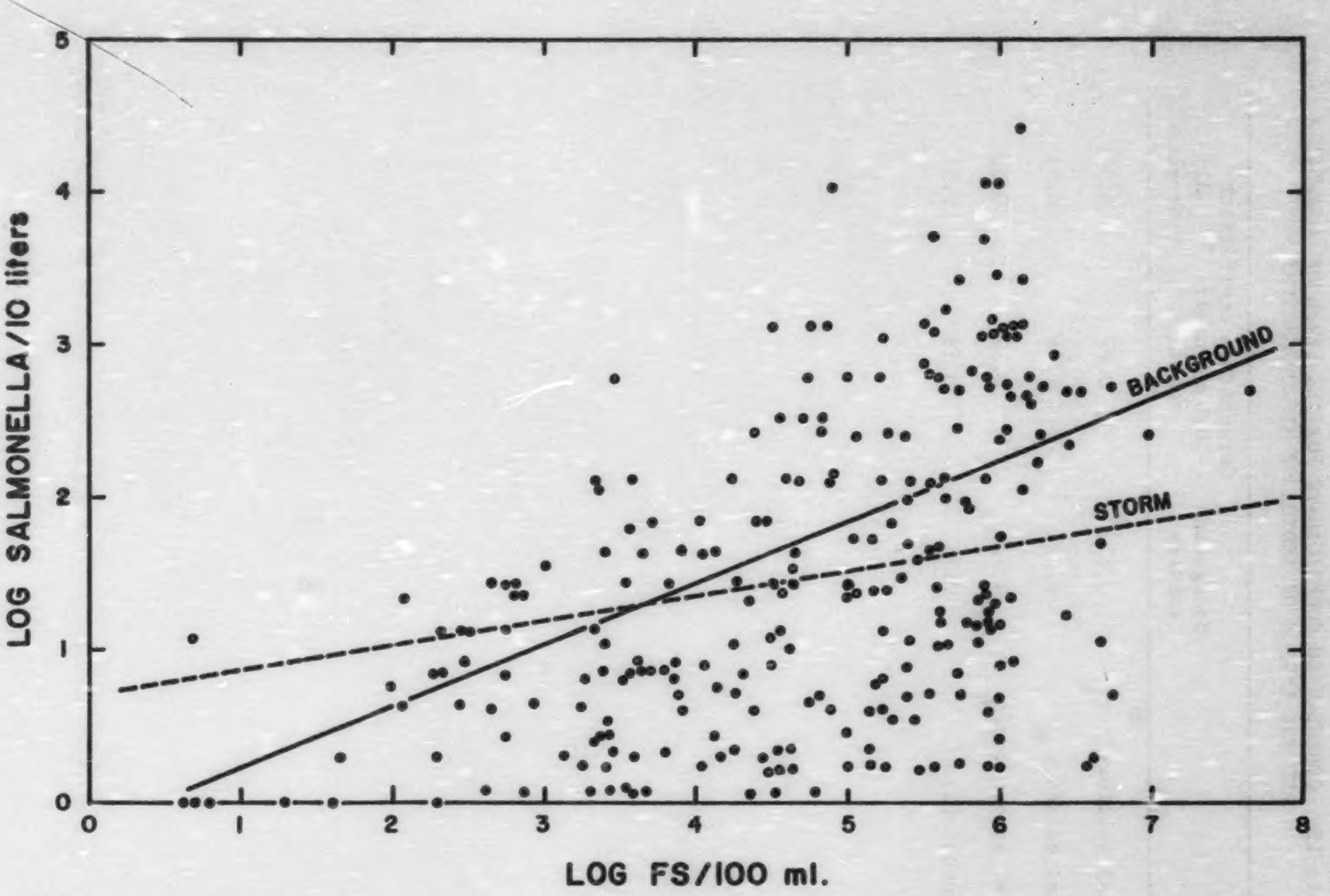


Figure 12. Relationship between fecal streptococci and *Salmonella* in background (solid point) and stormwater (open point) samples.

Table 15. CORRELATION COEFFICIENTS FOR THE LEVEL OF *SALMONELLA* AND THE LEVELS OF INDICATOR MICROORGANISMS

	Correlation coefficient Background samples	Urban runoff samples	All samples
Total coliform	0.67	0.49	0.54
Fecal coliform	0.72	0.36	0.59
Fecal streptococci	0.71	0.19	0.53
Enterococci	0.73	0.18	0.54

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PUBLIC HEALTH ASPECTS OF SURFACE WATERS IN THE WOODLANDS

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J. D. Moore,(2) D. Casserly(2)

The bacteriological content of surface waters in The Woodlands, Texas has been the subject of a multipurpose research investigation which was initiated in late November, 1973. Conceptually, that test site is a planned community covering approximately 20,000 acres and is located 35 miles north of Houston, Texas off Interstate Highway 45, and is being designed to ultimately house about 120,000 residents. An outline of the test area and the principal watercourses involved is included as Figure 1. It appeared at the onset of this investigation that the area would be ideal to study the effect on the bacteriological quality of stream waters by urbanization. None of the lakes indicated in Figure 1 are natural lakes but have been constructed for use as irrigation water sources or recreational waters. Since one of the objectives was to quantify the indicator bacteria and some pathogenic bacterial groups or species in the streams, the result of impounding the streamwaters became equally as important an objective.

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Determining stormwater runoff quality in Panther Branch and Bear Branch was another purpose of this investigation; depending on the viewpoint of the reader, perhaps the single most important purpose. Several research subsets of this latter purpose were developed. One parameter considered when judging water quality from a public health viewpoint is a series of indicator organism test results. Total coliform, fecal coliform, and fecal streptococci are currently in use for that reason and the ratio of fecal coliform to fecal streptococci has been given considerable research attention with regard to the source of the polluting bacterial groups. Analyses of those bacteria and their numerical ratios in stream waters in The Woodlands have been conducted for yet another reason. Point source wastewater discharges were believed to have been nonexistent in the test area. Therefore the data obtained from such an undeveloped watershed would be useful for comparison with other regions of the Country and evaluating the effectiveness of the fecal coliform (FC) to fecal streptococci (FS) ratio. Other tasks which were pursued included the quantification of pathogenic bacterial species or groups during periods of low flow in the streams as well as throughout storm event hydrographs and the determination of stormwater disinfection requirements employing chlorine and ozone. Three genera of pathogens have been quantified and include *Salmonella* sp., *Staphylococcus* sp., and *Pseudomonas aeruginosa*.

Total coliform bacteria were enumerated on m-Endo Agar (BBL) plates using Gelman membrane filters of 0.45μ porosity. Fecal coliform bacteria and fecal streptococci were plated on m-FC Agar (Difco) and m-Enterococcus Agar (BBL) respectively, also on membrane filters. Staphylococcus sp. colonies were enumerated using Vogel-Johnson Agar followed by testing most representative colonies for catalase positive reactions. Most plates yielded well over 90% catalase positive staphylococci. Pseudomonas aeruginosa was quantified on membrane filters using m-PA medium, the formula for which was provided by Dr. E. E. Geldreich, U.S.E.P.A., Cincinnati. Salmonella sp. quantification required the use of several media. Water samples were first plated onto Xylose Lysine Deoxycholate Agar (XLD) (BBL). All positive colonies were then transferred to Triple Sugar Iron Agar (TSI) (BBL) slants. From those, positives were further tested and confirmed using API test strips which employ screening via 20 biochemical reactions.

Initially, fifteen locations for low flow water quality monitoring were established in the test area. Those stations are indicated in Figure 2. Within the group, P-10* and P-30* were considered to be of prime importance due to their location above and below, respectively, the area in which construction was started following the initiation of this investigation. Data presented later in this treatise will refer to certain of those sample locations by code number.

*See next page.

*During the period of time that the data reported herein were developed some of the Woodlands area above station P-30 was under construction. A comparision of land use above station P-10 and above P-30 is as follows:

	Station P-10 (Upstream)	Station P-30 (Downstream)
Drainage Area, acres	16,050	21,606
% Impervious	1%	1%
Developed and/or under construction	1%	10%

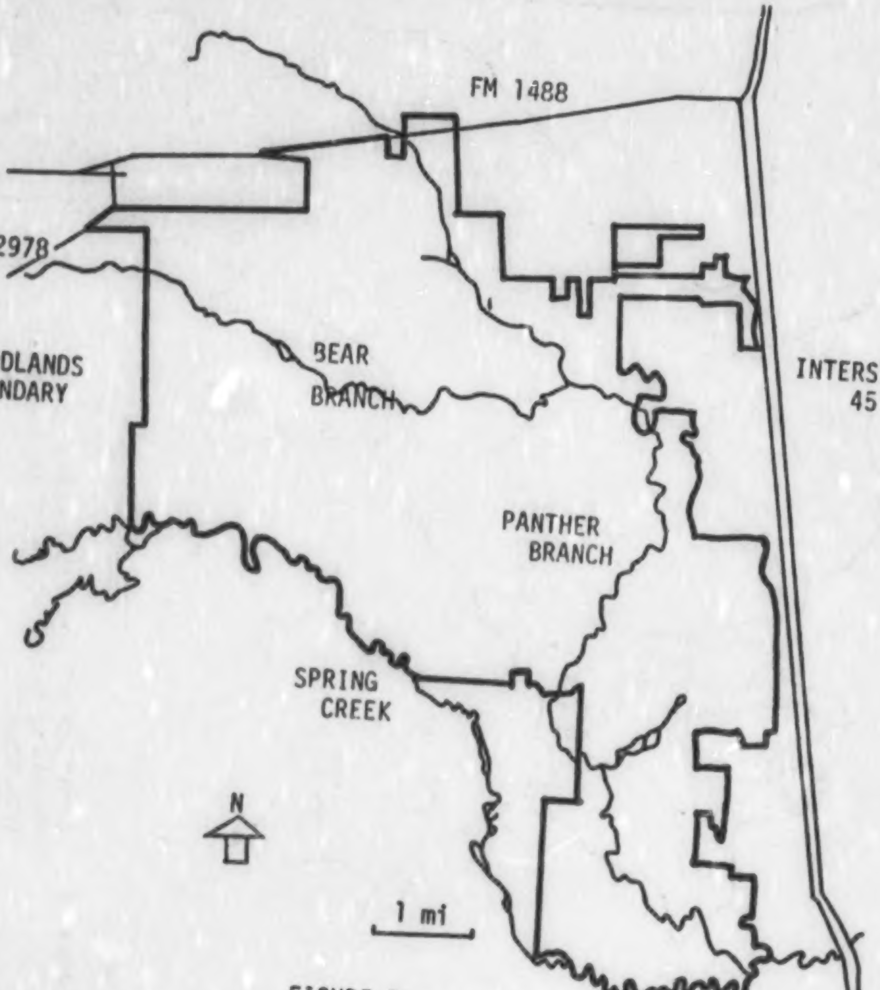

FM 1488
FM 2978
WOODLANDS BOUNDARY
BEAR BRANCH
INTERSTATE 45
PANTHER BRANCH
SPRING CREEK
N
1 mi

FIGURE 1
THE WOODLANDS AREA
MONTGOMERY COUNTY TEXAS

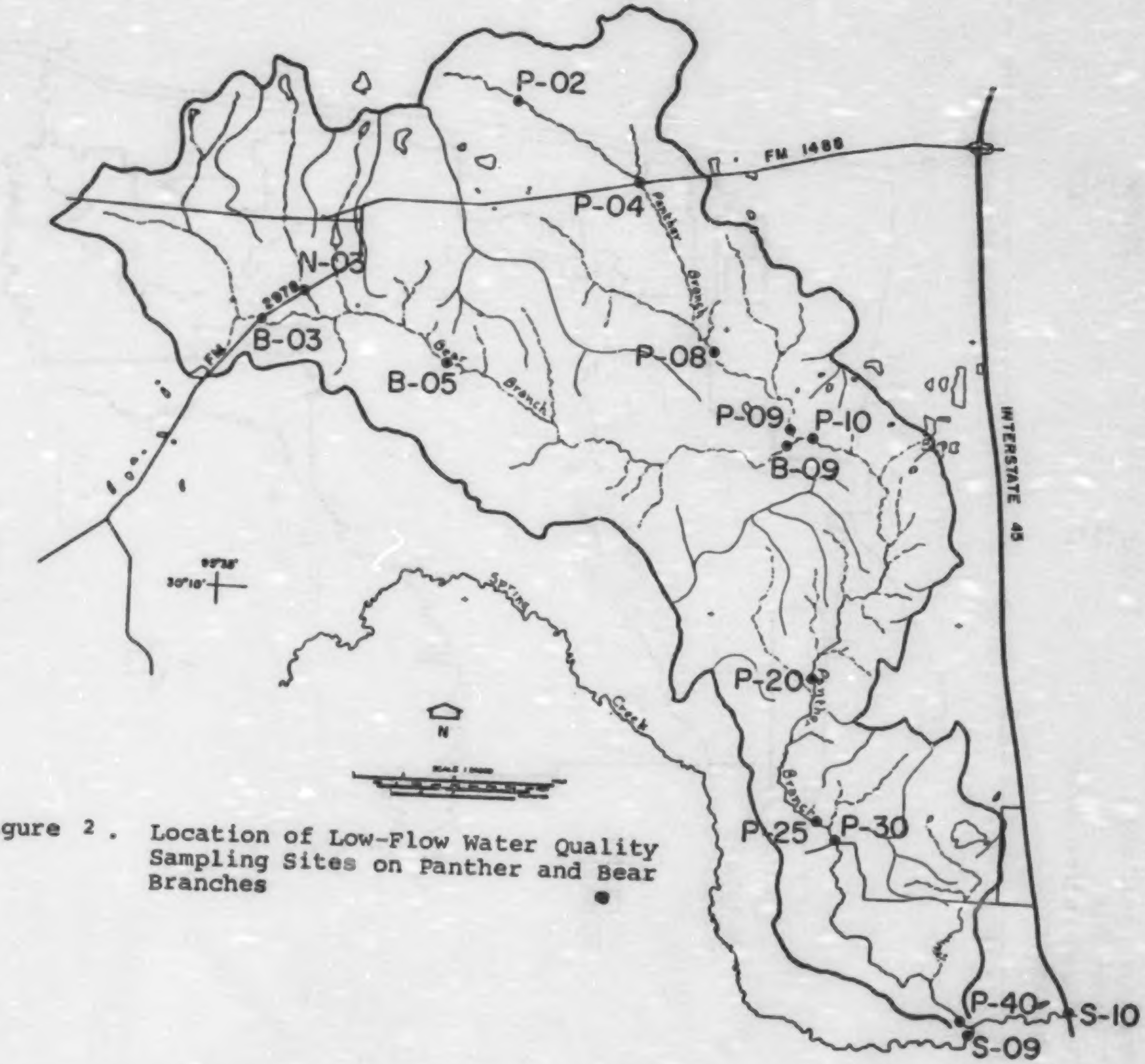

P-02
FM 1488
P-04
Panther Branch
N-03
2978
FM
B-03
B-05
Bear Branch
P-08
P-09
P-10
B-09
INTERSTATE 45
30°10'
Spring Creek
N
P-20
Panther Branch
P-25
P-30
P-40
S-10
S-09

Figure 2 . Location of Low-Flow Water Quality Sampling Sites on Panther and Bear Branches

One of the factors which had to be identified was the variability in density of the aforecited bacteria groups and/or species during low-flow with time. To this purpose a diurnal sampling scheme was initiated at stations P-10 and P-30 in September, 1974. The data developed from that project are listed in Tables 1 through 4, and represent sampling over approximately an 18-hour period of time. For all practial purposes the waters at both stations appeared to have been unfit for contact or noncontact recreation throughout the entire sampling period, if total coliform densities are the only parameter to be considered. Attention is directed to the high variability in concentrations in all organism counts. Those data suggest the possibility that typical approaches normally taken in judging water quality from a bacterial content viewpoint via one grab sample per month, for example, are, at best, only approximations. Mean values compared with standard deviations for each parameter in those Tables further the meaning of that suggestion. It is equally as important to take note of the overall increase in the FC/FS ratios and the FC/FS mean ratio between the two stations. The increase in densities of each occurred with distance downstream. The densities of Staphylococcus sp. and Pseudomonas aeruginosa definitely indicate a quality of water which posed a threat to the public health insofar as contact recreation was concerned.

During the course of this investigation, to date of the workshop, seven storm events have been monitored. To the purpose of brevity however, data for one representative event in the test site is included in this treatise. Naturally, all detailed data has been made available to USEPA personnel through monthly reports and year-end reports and may be obtained from the author. Tables 5 through 8 contain the bacteriological data for the storm event which was monitored from December 5 through December 9, 1974. Differentiation of water quality between the upper location's water quality and that at the downstream station (P-30) by examining the data for total coliform bacteria alone is obviously not possible. However, if one would compare the other indicators and pathogen densities over time, a pattern appears, and has appeared in other storm event data. An immediate increase in total coliform bacteria held, with highly variable densities throughout the major portion of the measured hydrograph. Fecal coliform and fecal streptococci, on the other hand, increased by several orders of magnitude during the initial phases of the storm event and decreased with time. They also exhibited higher densities in the downstream waters (P-30; Table 7) than at station P-10, upstream. FC/FS ratios were not consistent within the waters at either station throughout the entire storm event, i.e., if a basis is used for evaluating FC/FS ratios as to 4 (human, pollution), to <1 (animal pollution). On the other hand, Pseudomonas sp. concentrations were higher at station P-10 than at P-30 during the storm. Staphylococci may warrant special attention due to their behavior which is unlike that of any of the other groups. A general increase of from 700 or 800/100 ml to about 2,000/100 ml held throughout the early part of the storm event at P-10 whereas erratic variations, yet high concentrations occurred at station P-30 throughout the entire time of the storm event. A graphical representation of those parameters plus suspended solids and turbidity is presented in Figures 3 through 11. Initial runoff carries peak loading and/or concentrations of certain bacteria groups or species. Those graphs permit such a conclusion to be made as it becomes obvious when comparing points within the first twelve hours of the hydrograph. Among those factors which peaked prior to the hydrograph maximum flow rate were suspended solids, turbidity, and total coliform. Leaching from or washout of soil and forest floor cover is presumed to have been partially responsible for the peaks in bacterial concentrations following the hydrograph peak.

Disinfection requirements of stormwaters vary between times during the storm event as well as the concentration of suspended solids and other oxidant demanding (or combining) substances. Several experiments have been conducted to determine chlorine and ozone demand of varying qualities of water during storm events and of the requirements for disinfection to meet or exceed established or proposed standards. Tables 9 through 12 contain disinfectant data for preselected dosages of chlorine and ozone of discharge waters from Lake A (The Woodlands), the sample for which was taken at 0900 hrs. on March 13, 1975 during the storm event of 3/12-14/75. Lower initial (time zero) counts than are represented in earlier data are suspected to have been the result of that small impoundment (12.6 ac. surface; 90 ac-ft. vol.) acting as a settling basin. Values represented for "less than" some numerical figure imply a positive count but high turbidity impeded additional filtration, as has been the case in numerous instances. Ozonation was effective but not nearly so much within the first 30-minutes exposure as chlorine. Staphylococcus sp. demonstrated a marked capability to regenerate an appreciable population following ozonation. The total coliform group exhibited the greatest capacity for aftergrowth two days following exposure even up to 16 mg/l chlorine.

Over thirty composited soil samples have been collected from swales, forest floor, and near each sampling station (Figure 2) for the purpose of attempting to determine whether a principal source of bacteria in storm runoff was indeed the soil or whether unidentified point sources may have been in existence in the test area. Table 13 contains a partial listing of the sampled areas and bacteriological results from each. The results suggest that sufficient numbers of those listed bacteria groups or species exist in high humus soil to be carried out by rainfall leachate of high enough volume. To date no point sources of wastewater discharge have been identified in the upper reaches of the test site.

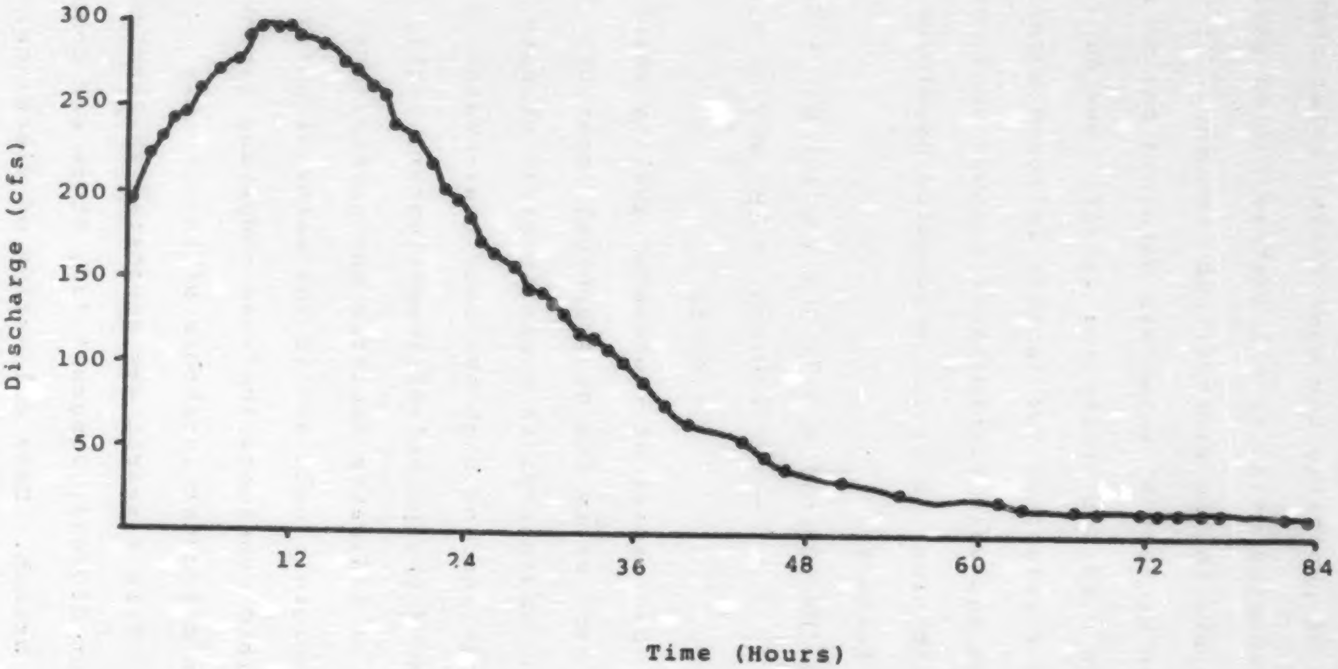
Currently, additional research is being directed to critical evaluations of the meaningfulness of the indicator bacteria counts and their related water quality factors. To the purpose of proposing the most useful relationship analyses have been conducted weighing all factors listed earlier. With 231 data points in the first attempt sequence, it was determined that the highest R^2 value was obtained for fecal streptococci. This was 0.82 and speaks for itself. Raw data did not produce as high a value but the factors followed normal distribution patterns and were mathematically sound when converted by log transformation. A useful expression developed from these data was as follows:

$$\begin{aligned}\text{Log }(\text{FS}/100\text{ ml}) = {} & 0.72\text{ Log }(\text{FC}/100\text{ ml}) + 0.002\ (\text{discharge}) \\ & + 0.003\ (\text{Turbidity}) + 0.002\ (\text{Susp. Solids}) \\ & + 0.612\end{aligned}$$

One aspect of stormwater quality which was not discussed earlier is its geographical location. The test site is located, obviously, in a semitropical climate with virtually no frost-line and high humidity year-round. It is, in the opinion of the principal investigator, highly conductive to the growth of indicator bacteria and perhaps even pathogens, given enough organic nutrient in the water or soil. This should be taken into consideration when comparing stormwater quality between different locations of the Country.

This research was supported in Part under USEPA Prime Contract R802433, Subgrant 733, Storm and Combined Sewer Technology Branch. Data collection of discharge, suspended solids, and turbidity by Rice University faculty and staff is hereby acknowledged.

Figure 3. Volume vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.



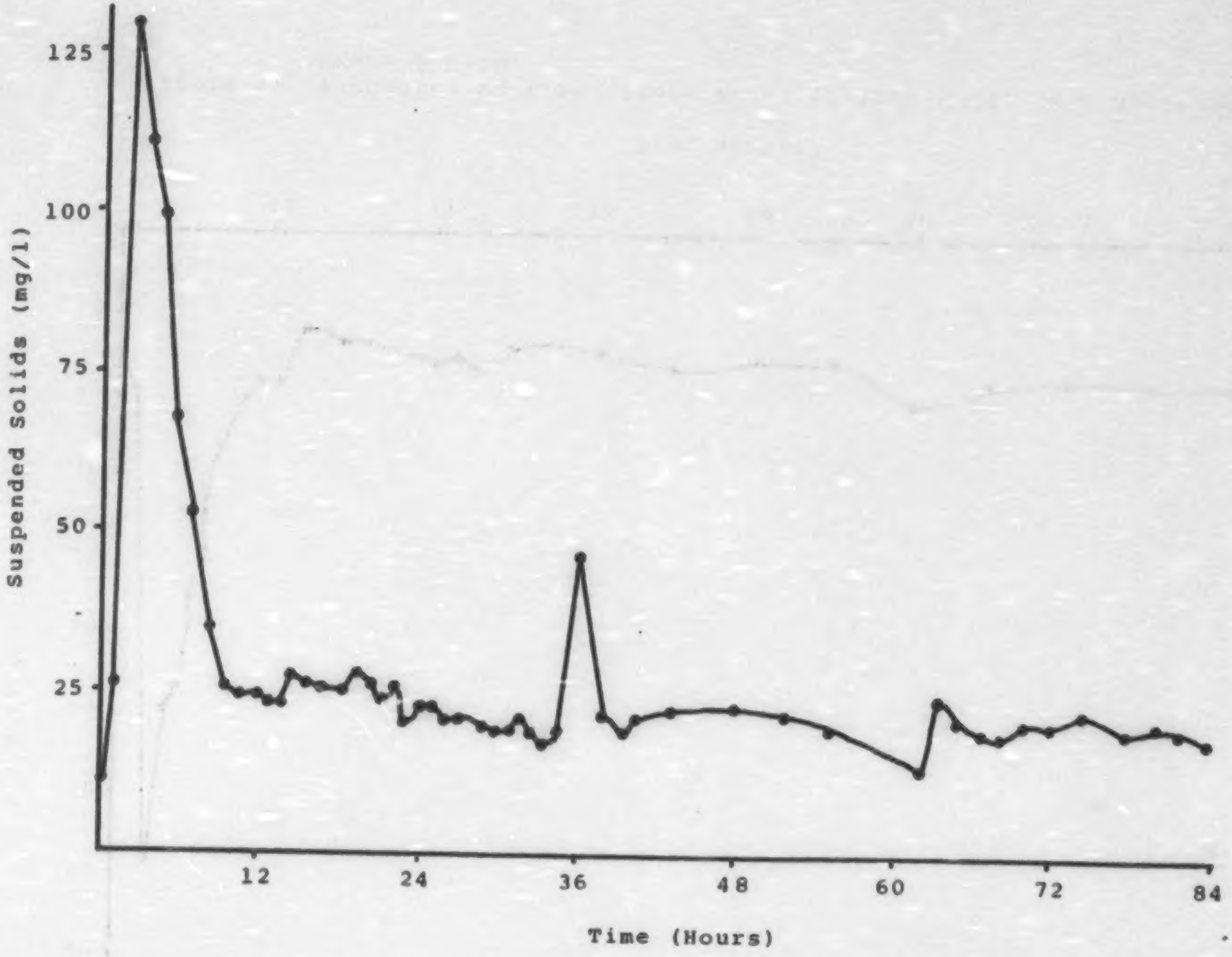


Figure 4. Suspended Solids vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.

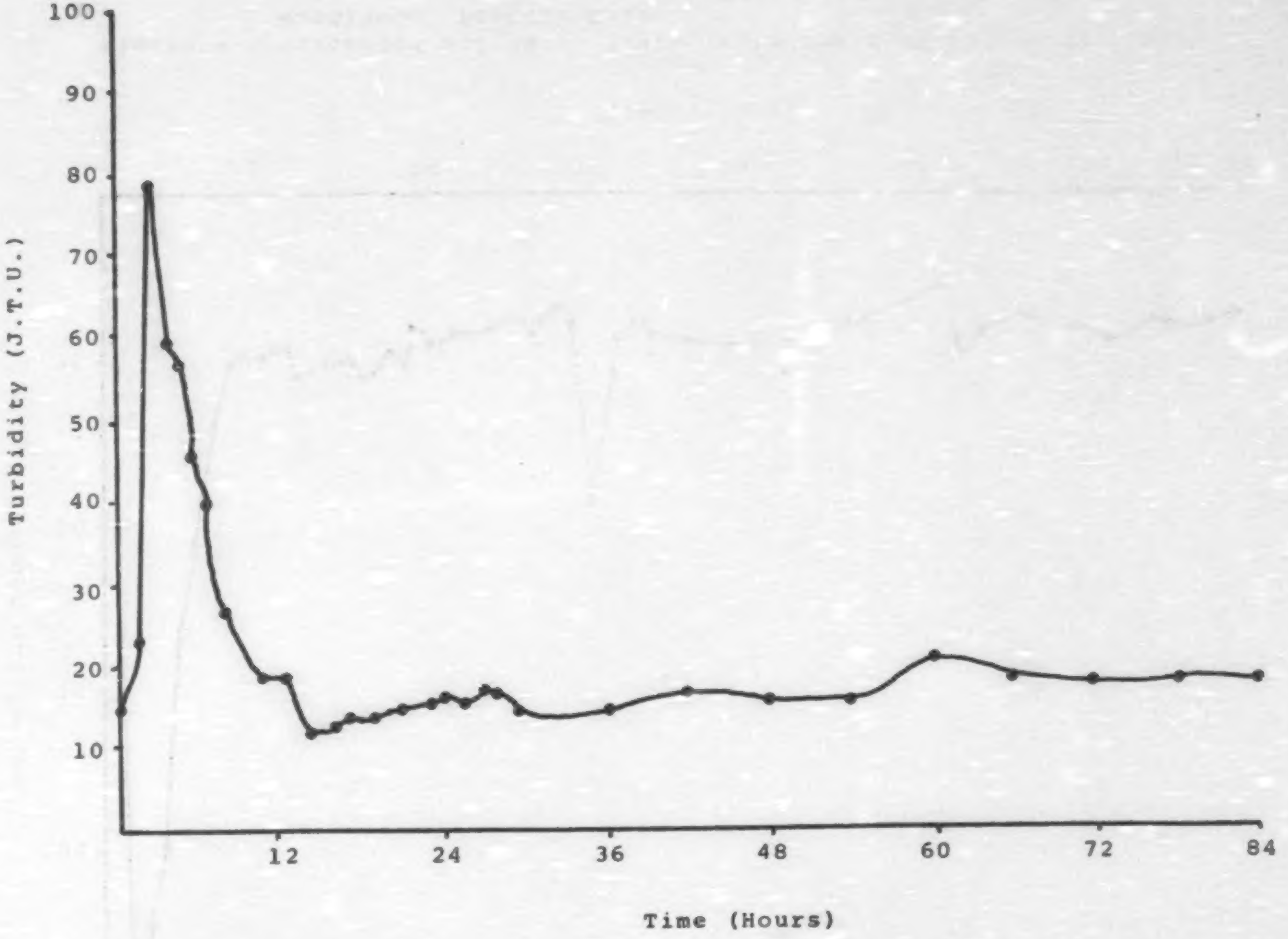
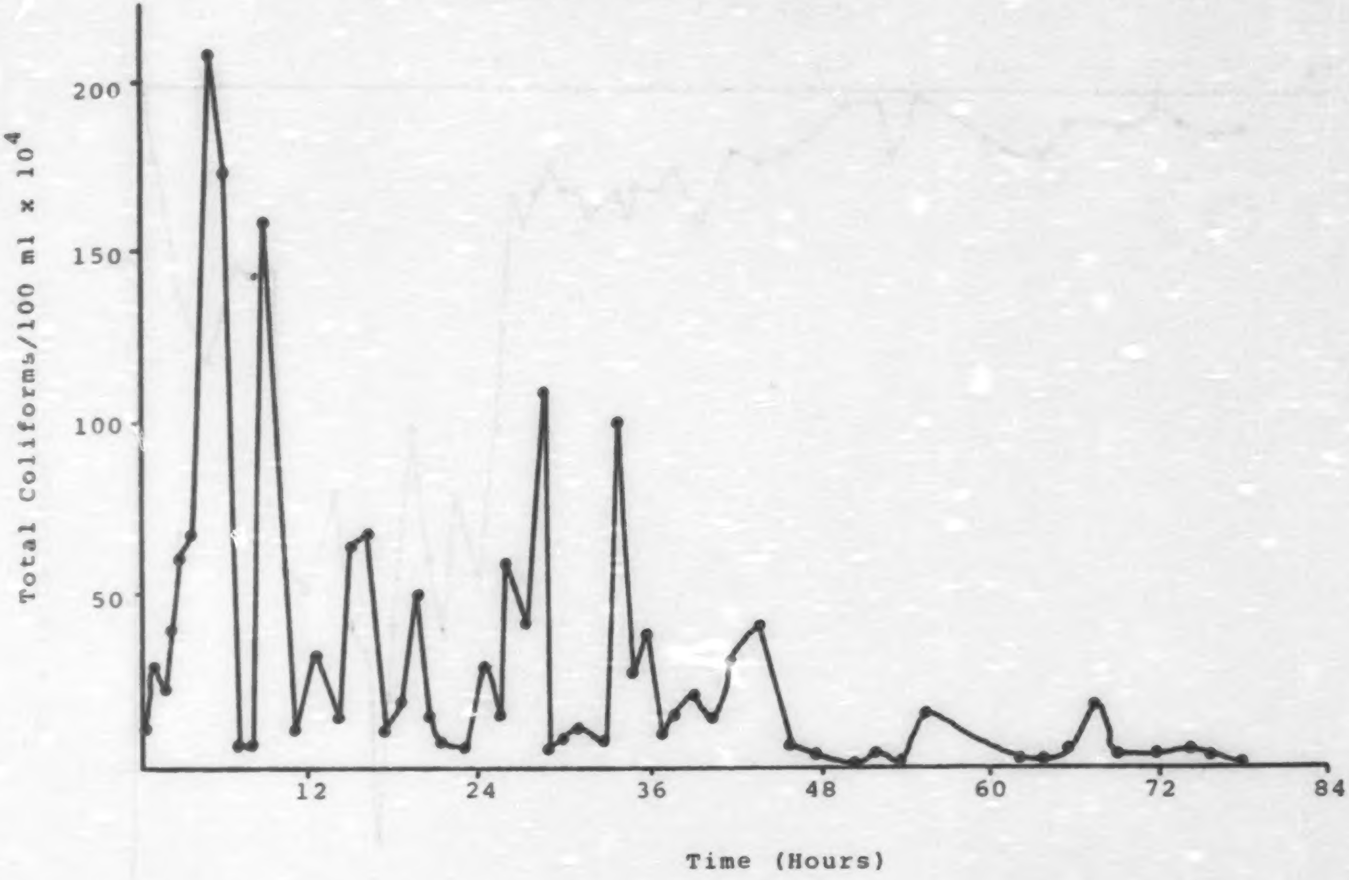


Figure 5. Turbidity vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.

Figure 6. Total Coliform vs Time; Storm Event of 12/5-9/74.
The Woodlands, Station P-10.



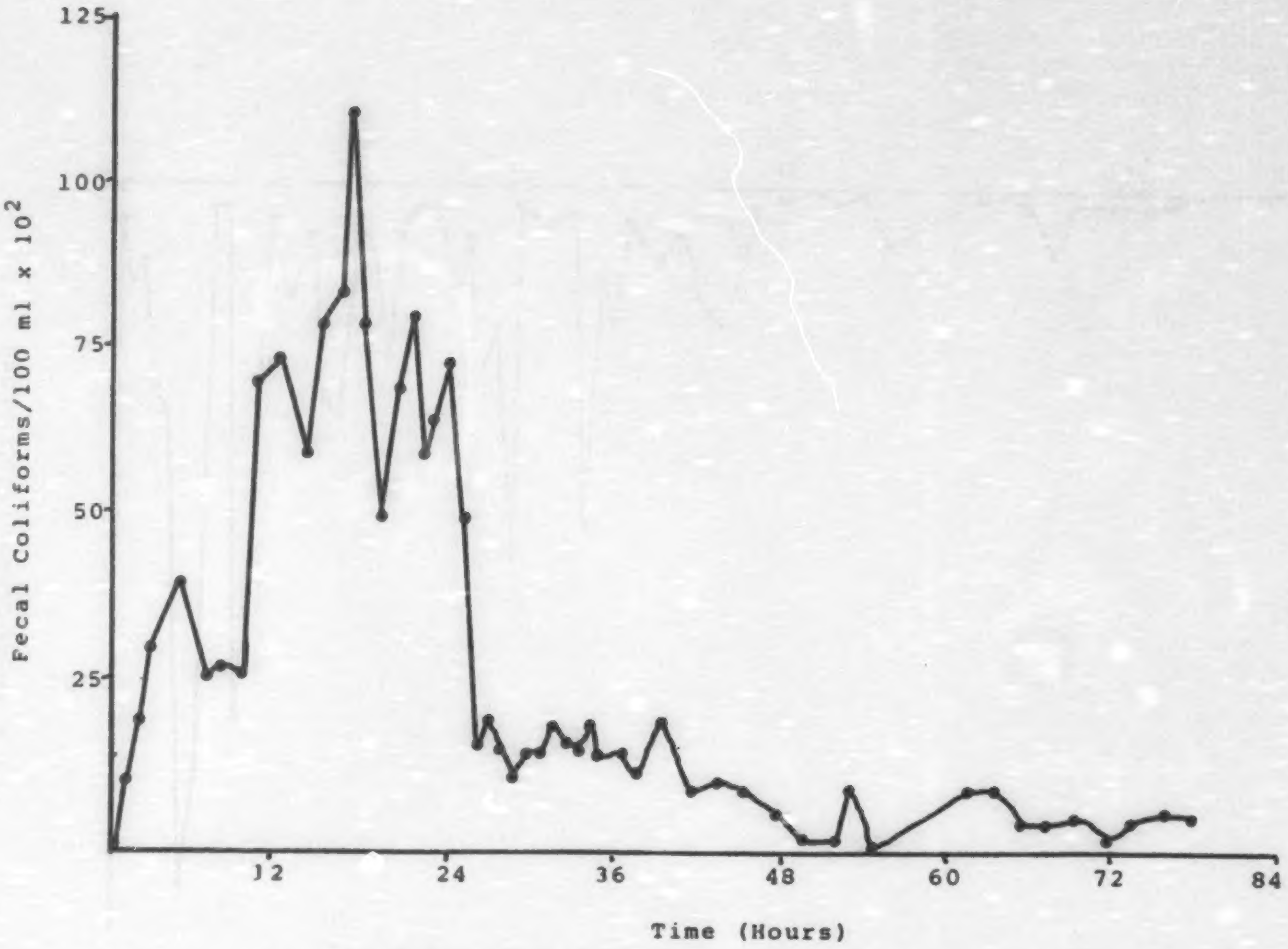


Figure 7. Fecal Coliform vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.

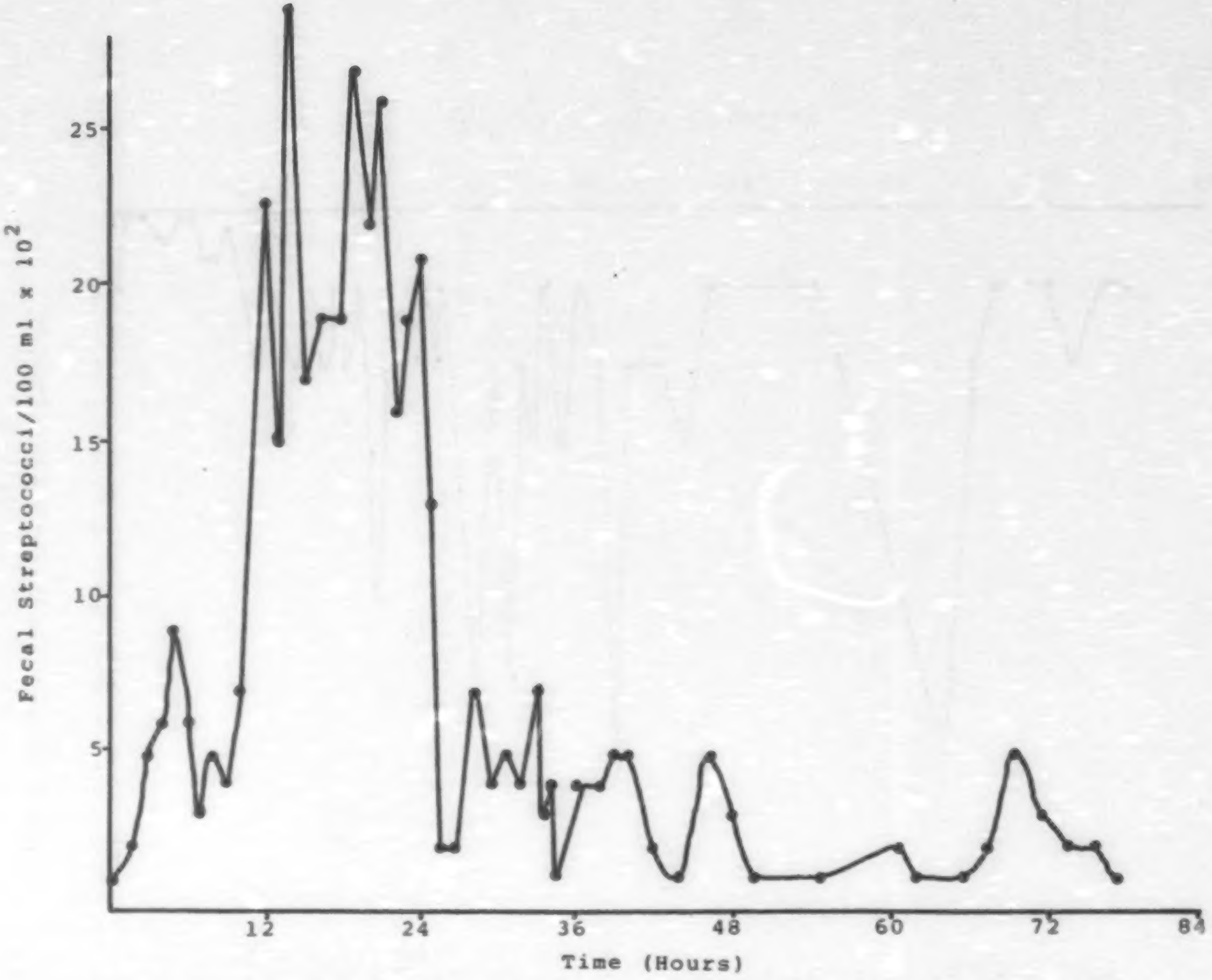
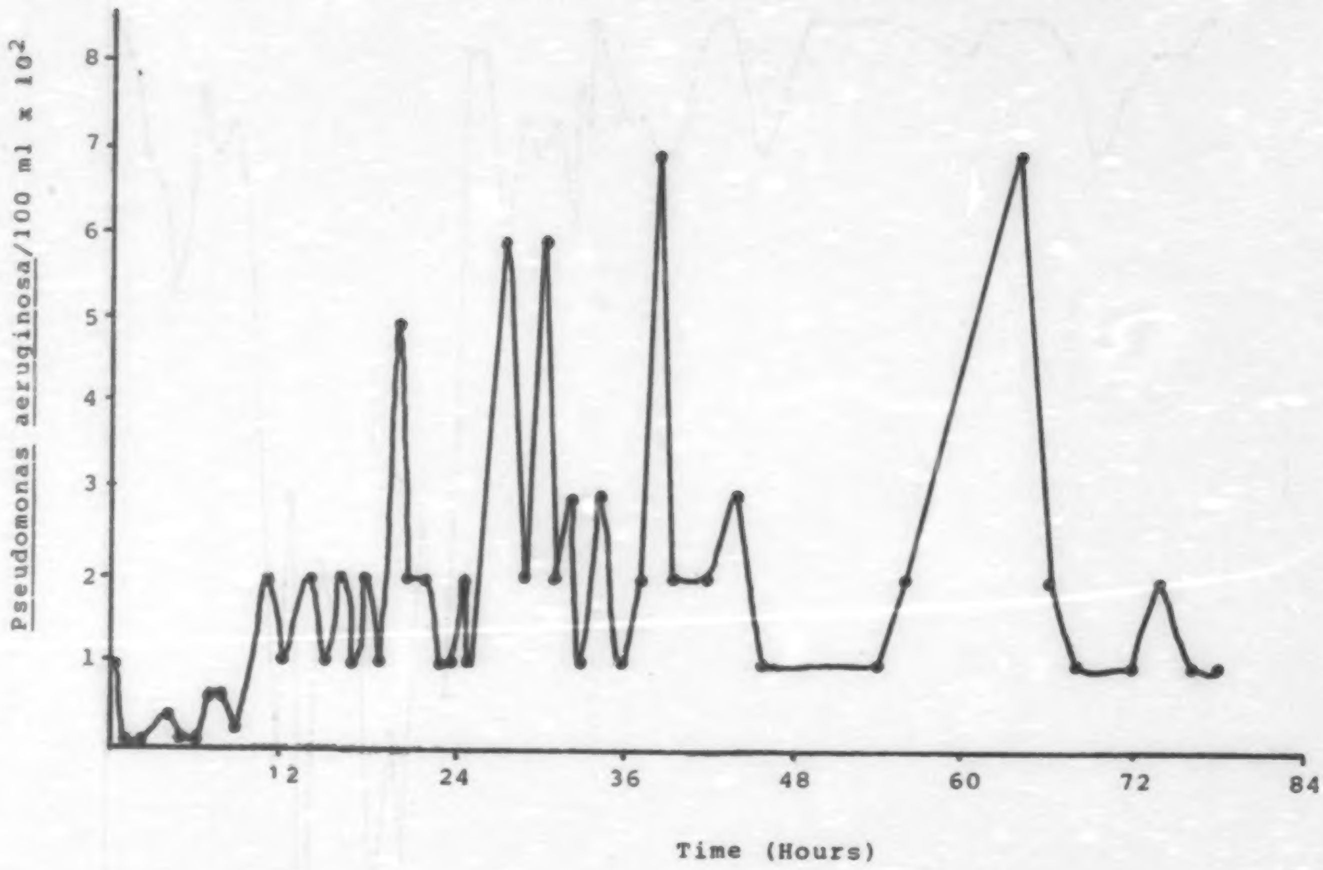


Figure 8. Fecal Streptococci vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.

Figure 9. _Pseudomonas_ _aeruginosa_ vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.



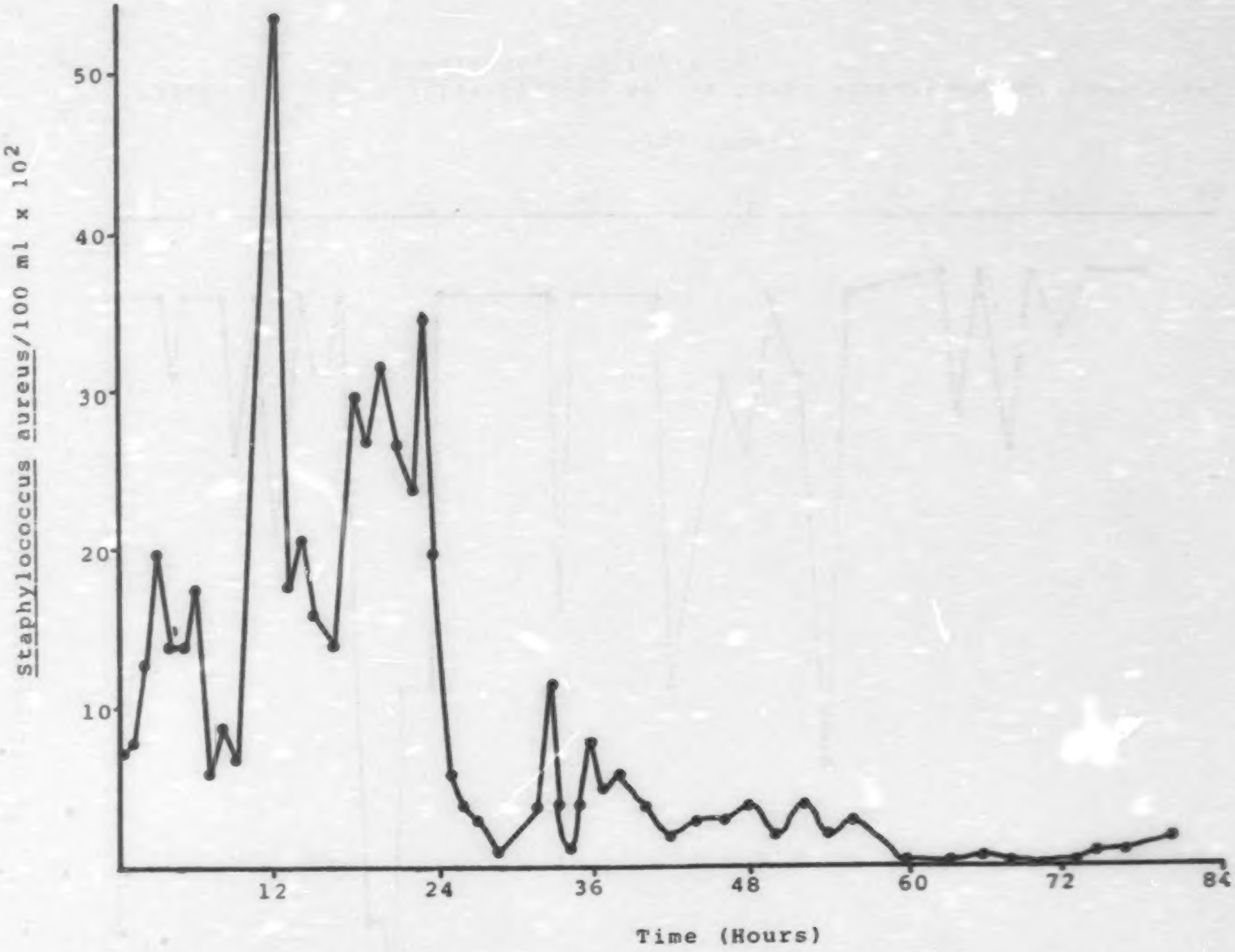


Figure 10. Staphylococcus aureus vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.

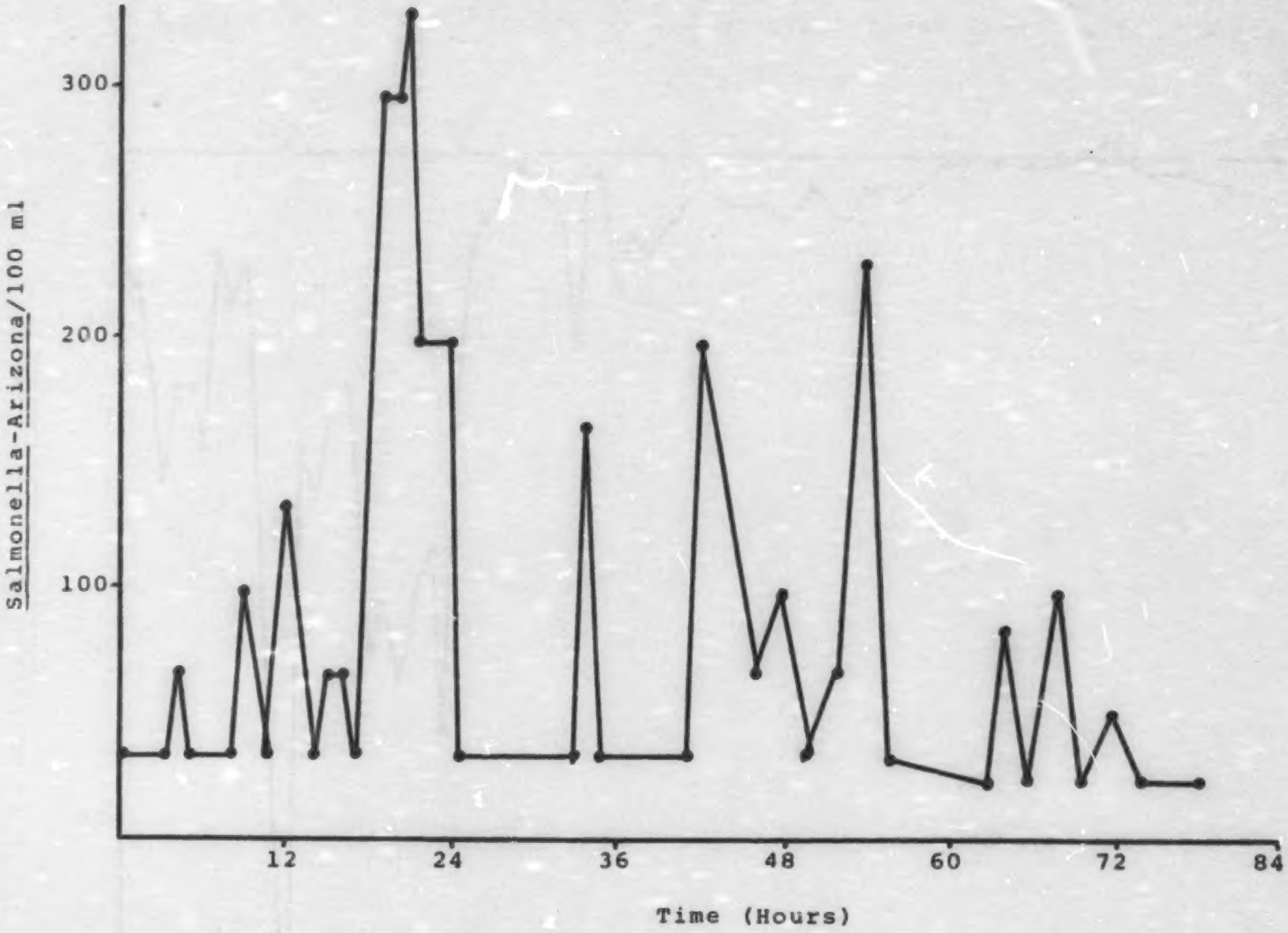


Figure 11. *Salmonella-Arizona* sp. vs Time; Storm Event of 12/5-9/74. The Woodlands, Station P-10.

Table 1

MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS IN THE WOODLANDS.
SAMPLE DATE: 9/21/74 - 9/22/74 (No./100 ml) DIURNAL STUDY.
STATION: P-10

Date & Time	Total Coliform,	Fecal Coliform,	Fecal Streptococci,	FC/FS
9/21/74				
5:45 PM	201,000	150	130	1.15
6:45	60,000	130	180	.72
7:45	70,000	210	190	1.11
8:45	51,000	310	250	1.24
9:50	56,000	220	290	.76
10:50	78,000	200	310	.65
11:50	51,000	190	300	.63
9/22/74				
12:50 AM	65,000	150	250	.60
1:50	48,000	180	310	.58
2:50	62,000	70	320	.22
3:50	55,000	80	260	.31
4:50	61,000	80	270	.29
5:55	22,000	10	200	.05
6:55	39,000	15	200	.08
7:55	6,000	50	170	.29
8:55	24,000	200	130	1.54
9:55	39,000	580	140	4.14
10:55	31,000	130	180	.72
n = 18	Σ=1,019,000	2,955	4,080	15.08
	$\bar{X}$= 56,611	164	227	.84
	σ= 40,478	127	65	.92

Table 2

RESULTS OF PATHOGEN ANALYSES OF SURFACE WATERS IN THE WOODLANDS.
SAMPLE DATE: 9/21/74 - 9/22/74 (NO./100 ml) DIURNAL STUDY.
STATION: P-10

Date & Time	Staphylococcus sp.	Pseudomonas aeruginosa	Salmonella sp.
9/21/74			
5:45 PM	300	30	200
6:45	510	<10	100
7:45	250	880	400
8:45	200	40	200
9:50	310	40	600
10:50	210	130	<100
11:50	400	250	300
9/22/74			
12:50 AM	450	450	500
1:50	200	150	100
2:50	350	750	1,500
3:50	400	650	400
4:50	90	950	1,600
5:55	200	80	100
6:55	150	50	<100
7:55	50	10	1,200
8:55	200	50	<100
9:55	350	60	<100
10:55	650	60	<100
n = 18	Σ=5,170	4,640	7,200 (n = 13)
	$\vec{X}$= 287	258	554
	σ= 195	325	532

Table 3

MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS: THE WOODLANDS.
SAMPLE DATE: 9/21/74 - 9/22/74 (No./100 ml) DIURNAL STUDY.
STATION: P-30

Date & Time	Total Coliform	Fecal Coliform	Fecal Streptococci	FC/FS
9/21/74				
4:50 PM	81,000	180	210	.86
5:50	78,000	230	210	1.10
6:50	83,000	270	270	1.00
7:50	69,000	160	150	1.07
8:50	83,000	220	350	.63
10:20	50,000	200	230	.87
11:20	112,000	290	240	1.21
9/22/74				
12:50 AM	96,000	280	250	1.12
1:55	100,000	160	180	.89
2:55	90,000	100	260	.38
3:55	101,000	150	180	.83
5:30	75,000	100	190	.53
6:30	79,000	160	100	1.60
7:30	51,000	350	70	5.00
8:30	48,000	420	90	4.67
9:30	76,000	230	140	1.64
10:30	115,000	220	130	1.69
11:30	55,000	200	220	.91
12:30 PM	79,000	180	110	1.64
1:30	71,000	180	300	.60
2:30	80,000	250	430	.58
3:30	122,000	200	560	.36
4:30	62,000	160	450	.36
n = 23	Σ=1,856,000	4,890	5,320	29.54
	$\bar{X}$= 80,696	213	213	1.28
	σ= 20,379	66	237	1.19

Table 4

RESULTS OF PATHOGEN ANALYSES OF SURFACE WATERS IN THE WOODLANDS.
SAMPLE DATE: 9/21/74 - 9/22/74 (No./100 ml) DIRUNAL STUDY.
STATION: P-30

Date & Time	Staphylococcus sp.	Pseudomonas aeruginosa	Salmonella sp.
9/21/74			
4:50 PM	650	180	100
5:50	550	260	600
6:50	1,100	340	700
7:50	500	110	100
8:50	2,000	190	<100
10:20	950	190	200
11:20	1,500	90	200
9/22/74			
12:50 AM	750	170	400
1:55	250	180	300
2:55	250	150	200
3:55	150	110	100
5:30	650	90	400
6:30	100	100	<100
7:30	700	60	<100
8:30	610	50	<100
9:30	1,000	160	<100
10:30	400	120	<100
11:30	2,500	110	<100
12:30 PM	800	130	<100
1:30	650	150	100
2:30	500	370	<100
3:30	400	540	300
4:30	1,250	400	200
n = 23	Σ=18,210	4,250	3,900 (n = 14)
	$\bar{X}$= 792	185	279
	σ= 582	123	188

Table 5

MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-10.

(No./100 ml)

Date & Time	Total Coliform	Fecal Coliform	Fecal Streptococci	FC/FS
12/5/74				
2130	$3x10^4$	100	100	1.0
2200	$30x10^4$	1,100	200	5.5
2300	$23x10^4$	1,900	500	3.8
2400	$61x10^4$	3,100	600	5.2
12/6/75				
0100	$69x10^4$	3,500	900	3.9
0200	$210x10^4$	4,100	600	6.8
0300	$174x10^4$	3,400	300	11.3
0400	$7x10^4$	2,600	500	5.2
0500	$7x10^4$	2,800	400	7.0
0600	$161x10^4$	2,700	700	3.8
0730	$11x10^4$	7,100	1,800	3.9
0830	$32x10^4$	7,400	2,300	3.2
1015	$15x10^4$	5,900	1,500	3.9
1115	$66x10^4$	7,900	2,900	2.7
1215	$68x10^4$	8,300	1,700	4.9
1315	$11x10^4$	11,500	1,900	6.0
1415	$19x10^4$	7,800	1,900	4.1
1515	$52x10^4$	5,000	2,700	1.8
1615	$15x10^4$	6,800	2,200	3.1
1715	$8x10^4$	8,200	2,600	3.1
1815	$6x10^4$	5,900	1,600	3.7
1919	$7x10^4$	6,600	1,900	3.5
2015	$30x10^4$	7,300	2,100	3.5
2115	$15x10^4$	5,000	1,300	3.8
2145	$63x10^4$	1,500	200	7.5
2245	$42x10^4$	1,900	200	9.5

Table 5 (Cont'd)

MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-10.

(No./100 ml)

Date & Time	Total Coliform	Fecal Coliform	Fecal Streptococci	FC/FS
2345	111x10^4	1,600	700	2.3
12/7/74				
0045	5x10^4	1,100	400	2.7
0145	7x10^4	1,500	400	3.7
0245	11x10^4	1,600	500	3.2
0345	9x10^4	1,900	400	4.7
0445	7x10^4	1,700	700	2.4
0545	101x10^4	1,600	300	5.3
0645	27x10^4	1,900	400	4.7
0715	39x10^4	1,400	100	14.0
0815	9x10^4	1,500	400	3.7
0915	15x10^4	1,500	400	3.7
1045	21x10^4	1,200	500	2.4
1245	14x10^4	1,900	500	3.8
1445	31x10^4	900	200	4.5
1645	41x10^4	1,100	100	11.0
1845	5x10^4	900	500	1.8
2045	3x10^4	600	300	2.0
2245	1x10^4	200	100	2.0
12/8/74				
0045	4x10^4	200	100	2.0
0245	1x10^4	800	100	8.0
0445	16x10^4	100	200	0.5
0545	5x10^4	500	200	2.5
1115	2x10^4	800	100	8.0
1315	1x10^4	800	100	8.0
1515	6x10^4	300	100	3.0
1715	23x10^4	300	200	1.5

Table 5 (Cont'd)

MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-10.

(No./100 ml)

Date & Time	Total Coliform	Fecal Coliform	Fecal Streptococci	FC/FS
1915	$3x10^4$	500	500	5.0
2115	$4x10^4$	200	300	0.7
2315	$5x10^4$	300	200	1.5
12/9/74				
0115	$4x10^4$	600	200	3.0
0315	$1x10^4$	500	100	5.0

Table 6.

PATHOGEN ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-10.

(No./100 ml)

Date & Time	*Pseudomonas aeruginosa*	*Staphylococcus* sp.	*Salmonella* sp.
12/5/74			
2130	100	700	33
2200	10	800	<33
2300	10	1,300	<33
2400	20	2,000	33
12/6/74			
0100	40	1,400	66
0200	10	1,400	<33
0300	10	1,500	<33
0400	60	600	<33
0500	60	900	<33
0600	20	700	99
0730	200	2,800	33
0830	100	5,400	132
1015	200	1,800	33
1115	100	2,100	66
1215	200	1,600	66
1315	100	1,400	33
1415	200	3,000	165
1515	100	2,700	297
1615	500	3,200	297
1715	200	2,700	330
1815	200	2,400	165
1915	100	3,500	198
2015	100	2,000	198
2115	200	2,800	33
2145	100	600	<33
2245	300	400	<33

Table 6 (Cont'd)

PATHOGEN ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-10.

(No./100 ml)

Date & Time	Pseudomonas aeruginosa	Staphylococcus sp.	Salmonella sp.
2345	600	300	<33
12/7/74			
0045	300	100	33
0145	200	200	33
0245	600	300	<33
0345	200	400	33
0445	300	1,164	<33
0545	10,100	400	165
0645	300	100	<33
0715	200	400	<33
0815	100	800	33
0915	200	500	<33
1045	700	600	33
1245	200	400	<33
1445	200	200	198
1645	300	300	132
1845	100	300	66
2045	100	400	99
2245	100	200	<33
12/8/74			
0045	100	400	66
0245	100	200	231
0445	200	300	<33
0545	500	<10	<25
1115	600	10	<25
1315	700	30	75
1515	200	140	<25
1715	100	<10	100

Table 6 (Cont'd)

PATHOGEN ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-10.

(No./100 ml)

Date & Time	Pseudomonas aeruginosa	Staphylococcus sp.	Salmonella sp.
1915	100	<10	25
2115	100	<10	50
2315	200	100	<25
12/9/74			
0115	100	100	<25
0315	100	200	<25

Table 7

MICROBIOLOGICAL ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-30.

(No./100 ml)

Sample No.	Total Coliform	Fecal Coliform	Fecal Streptococci	FC/FS
1	3×10^4	400	100	4.0
3	15×10^4	5,200	500	10.4
5	104×10^4	6,900	2,200	3.1
7	5×10^4	12,800	3,200	4.0
9	55×10^4	18,900	3,600	5.2
10	34×10^4	11,100	17,500	0.6
11	5×10^4	14,800	1,800	8.2
12	73×10^4	1,200	14,200	0.1
13	26×10^4	13,700	3,100	4.4
14	27×10^4	7,700	5,200	1.5
15	4×10^4	14,600	2,700	5.4
16	110×10^4	9,200	13,000	0.7
17	112×10^4	16,300	2,800	5.8
19	57×10^4	8,300	1,900	4.4
20	311×10^4	34,000	6,100	5.6
22	9×10^4	10,400	3,800	2.7
24	3×10^4	800	3,900	0.2

Table 8

PATHOGEN ANALYSIS OF SURFACE WATERS IN THE WOODLANDS: STORM EVENT OF 12/5/74 - 12/9/74. STATION P-30.

(No./100 ml)

Sample No.	*Pseudomonas aeruginosa*	*Staphylococcus* sp.	*Salmonella* sp.
1	100	13,700	33
3	800	1,500	33
5	500	3,500	66
7	400	800	264
9	300	900	198
10	1,000	2,500	33
11	200	1,500	66
12	1,600	6,300	66
13	100	1,500	165
14	11,200	11,600	231
15	100	1,400	132
16	8,500	5,800	33
17	100	1,800	66
19	100	2,100	33
20	5,100	7,900	66
22	2,500	3,300	231
24	1,000	3,100	429

Table 9

DISINFECTION REQUIREMENTS OF STORM WATER IN THE WOODLANDS. SAMPLE TAKEN DURING STORM EVENT OF 3/12/75 - 3/14/75. STATION: LAKE A; 0900 3/13/75 (No./100 ml).

	Chlorination (mg/l)		
Time	Total Coliform	Fecal Coliform	Fecal Streptococci
t=0 hrs.	160×10^3	100	4,000
30 min.			
Control	157×10^3	<10	4,000
2	200	<10	3,980
4	<100	<10	<10
8	<100	<10	<10
16	<100	<10	<10
24 hrs.			
Control	8×10^3	10	3,500
2	<100	<10	10
4	<100	<10	<10
8	<100	<10	<10
16	<100	<10	<10
48 hrs.			
Control	<100	<10	1,490
2	<100	<10	<10
4	<100	<10	<10
8	<100	<10	<10
16	<100	<10	<10
96 hrs.			
Control	26.2×10^3	<10	520
2	600	<10	<10
4	380×10^3	<10	<10
8	60×10^3	<10	<10
16	113×10^3	<10	<10
8 Days			
Control	24×10^5	<10	50
2	8×10^4	<10	<10
4	19×10^5	<10	<10
8	36×10^4	<10	<10
16	5×10^5	<10	<10

Table 10

DISINFECTION REQUIREMENTS OF STORM WATER IN THE WOODLANDS.
SAMPLE TAKEN DURING STORM EVENT OF 3/12/75 - 3/14/75.
STATION: LAKE A; 0900 3/13/75 (No./100 ml).

Time	Staphylococcus aureus	Pseudomonas aeruginosa	Salmonella sp.
Chlorination (mg/l)			
t=0 hrs.	180	40	<25
30 min.			
Control	160	10	<25
2	60	10	<25
4	10	10	<25
8	<10	<10	<25
16	<10	10	<25
24 hrs.			
Control	80	<10	<25
2	50	<10	<25
4	10	<10	<25
8	10	<10	<25
16	<10	<10	<25
48 hrs.			
Control	460	10	<25
2	120	<10	<25
4	<10	<10	<25
8	160	<10	<25
16	40	<10	<25
96 hrs.			
Control	10	<10	<25
2	300	<10	<25
4	1,280	<10	<25
8	20	<10	<25
16	<10	<10	<25
8 Days			
Control	80	<10	<25
2	110	<10	<25
4	150	<10	<25
8	50	<10	<25
16	<10	<10	<25

Table 11

DISINFECTION REQUIREMENTS OF STORM WATER IN THE WOODLANDS.
SAMPLE TAKEN DURING STORM EVENT OF 3/12/75 - 3/14/75.
STATION: LAKE A; 0900 3/13/75 (No./100 ml).

Ozonation (mg/1)

Time	Total Coliform	Fecal Coliform	Fecal Streptococci
t=0 hrs.	219×10^2	160	3,500
30 min.			
Control	157×10^2	130	3,100
2	102×10^2	100	100
4	647×10^2	40	<10
8	114×10^2	20	100
16	26×10^2	90	<10
24 hrs.			
Control	1×10^2	20	600
2	<100	20	900
4	<100	40	910
8	<100	110	1,070
16	<100	90	60
48 hrs.			
Control	153×10^2	<10	200
2	232×10^2	<10	100
4	57×10^2	<10	<100
8	400×10^2	<10	<100
16	575×10^2	<10	<100
96 hrs.			
Control	146×10^2	10	400
2	124×10^2	<10	200
4	126×10^2	20	600
8	217×10^3	10	400
16	400×10^3	10	<100
8 Days			
Control	17×10^4	<10	<100
2	5×10^4	<10	<100
4	1×10^4	<10	<100
8	5×10^4	<10	<100
16	83×10^4	<10	<100

Table 12

DISINFECTION REQUIREMENTS OF STORM WATER IN THE WOODLANDS. SAMPLE TAKEN DURING STORM EVENT OF 3/12/75 - 3/14/75. STATION: LAKE A; 0900 HRS. 3/13/75 (No./100 ml). PATHOGEN ANALYSIS.

Ozonation (mg/1)

Time	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella* sp.
t=0 hrs.	160	50	<25
30 min.			
Control	110	40	<25
2	290	30	<25
4	90	<10	<25
8	110	60	<25
16	60	40	<25
24 hrs.			
Control	220	40	<25
2	250	50	<25
4	110	50	<25
8	180	20	<25
16	160	20	<25
48 hrs.			
Control	540	100	<25
2	840	150	<25
4	790	<10	<25
8	740	100	<25
16	50	110	<25
96 hrs.			
Control	1,350	<10	<25
2	1,100	<10	<25
4	740	<10	<25
8	1,090	<10	<25
16	1,500	10	<25
8 Days			
Control	60	<10	<25
2	150	<10	<25
4	90	<10	<25
8	170	<10	<25
16	50	<10	<25

Table 13

INDICATOR AND PATHOGEN CONCENTRATIONS IN SOILS AT FIVE LOCATIONS IN THE WOODLANDS.

SAMPLE DATE: 10/20/74

(No./gram)

Location	Total Coliform	Fecal Coliform	Fecal Streptococci	FC/FS
Woods at Lake B	124,000	35	100	0.3
Woods at Lake A	68,000	250	240	1.0
Woods South of Conference Center	58,000	250	100	2.5
Ditch on Woodlands Blvd.	15,600	90	10	9.0
Swale Entering Lake B	9,400	210	390	0.5

Location	Pseudomonas sp.	Staphylococcus sp.	Salmonella sp.
Woods at Lake B	300	50	<30
Woods at Lake A	90	160	<30
Woods South of Conference Center	100	150	30
Ditch of Woodlands Blvd.	230	600	<30
Swale Entering Lake B	120	250	30

EXPERIENCES WITH RECOVERY OF VIRUSES FROM STORM WATER

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ABSTRACT

A study was made to determine the feasibility of using the Aquella virus concentrator (Carborundum Co.) to detect live animal viruses in real and simulated combined storm overflows. Storms in two locations are being investigated presently--Syracuse, N. Y. and Baltimore, Md. In Syracuse 55 gallon samples are collected, concentrated and stored; Baltimore samples of 5 to 20 gallons are shipped by bus to Syracuse for analysis. Analysis of virus survival subsequent to shipping (Table 1) showed that little inactivation occurred as a result of thermal shock since viruses in TPB or sewage plant influent were not appreciably reduced in titer. However, Gwynns Falls creek water seeded with laboratory viruses commonly showed reductions of 50 to 60 per cent. This is not a progressive inactivation since seeded creek water maintained its titer for nine days when stored at 4C (Table 2). Other work showed that the creek water inactivated seed virus within a few minutes after the virus was diluted in it and before any thermal inactivation could take place.

Two concentration techniques have been employed to adsorb viruses preferentially to Cox epoxy fiberglass filters (Figure 1). Picornaviruses can tolerate a combination of low pH for adsorption (pH 3.5) and high pH for elution (pH 11.5). This effectively eliminates adenoviruses and reoviruses. To preserve them, adsorption is at pH 4.5 and elution, at pH 9.0.

Picornaviruses are also adsorbed under these conditions and they are eliminated routinely by the addition of antisera. More than 60 percent of the isolates in combined storm overflow are polioviruses--probably vaccine strains--and it is necessary to suppress them since they quickly overgrow most other viruses. Benzimidazoles suppress many single stranded RNA viruses but have little effect on DNA adenoviruses or double-strand RNA reoviruses. The latter two are easily differentiated on the basis of CPE and cytopathic inclusions.

Isolations have been made principally on HEp-2, W1-38 and pig kidney PK15 cells. More recently BGM cells and FLOW Labs #2000 fetal lung cells have been studied for comparative plating efficiency. It is anticipated that BGM cells will be the cell line of choice for most water-borne viruses of human origin. Pig kidney PK15 cells did not prove to be a broad adenovirus indicator line as we had anticipated and were decidedly inferior to HEK cells.

General operating experience with the Aquella virus concentrator suggests that recovery of small numbers of viruses from large volumes of storm overflow is possible but the efficiency is more in the range of 30 per cent, particularly when a combination of heavy silt and high organic content is encountered. The number of 50 gallon samples which can be processes completely in one working session is limited to about two/day. As a consequence we either store 50 gallon samples at 4C or else concentrate the sample to the first elution step and maintain the sample while we process other large samples. Enteroviruses survive the holding processes well but the data concerning longevity of adenoviruses and reoviruses are still incomplete.

Table 3 summarizes the qualitative aspects of storm samples which have been studies to date. Viruses have been found in nearly all sewage samples in both the raw sewage and the chlorinated effluents. Poliovirus is the most frequent but other viruses are usually in the same sample of fecal polluted water. (The values are based on assays which directly examined 20 per cent of the whole concentrated sample). Despite the obvious difference in sample sizes, Syracuse storm overflow and Baltimore storm overflow do not differ greatly in the detection frequency. This probably is fortuitous, but suggests that the Aquella concentrator might be redesigned to handle more samples at lower expense.

A limited number of Syracuse storms have been analyzed at the Maltbie Street treatment facility. Viruses were detected in storm overflows with one sample being particularly marked (7/3/74). Experience with the chlorine dioxide treatment is still too limited to make any real conclusions concerning its effectiveness against naturally occurring viruses. Some difficulties in logistics were experienced when samples were first being acquired and these samples tended to be collected at widely spaced intervals in the storm. Samples collected later in the season were pumped into drums and are more representative of water for any given period in the storm.

Although the project is still in its early stage, viruses have been recovered from both dry flow of urban Baltimore creeks and from storm overflows (Table 5). The viruses are barely at the detectable level and the identification of the serotypes of the viruses is still in progress. As might be anticipated, dry season flows tend to show higher titers than storm flows. Those areas with no intentional sanitary discharge such as Northwood have proved to be negative so far. Those areas with overflow discharge, Howard Park, Jones Falls Storm Outlet, and Bush Street, reflect fecal discharge.

Fig. 1. Identification and quantification of viruses in storm waters after concentrating them with the Aquella instrument. Cell culture: HEp-2 and BGM cells.

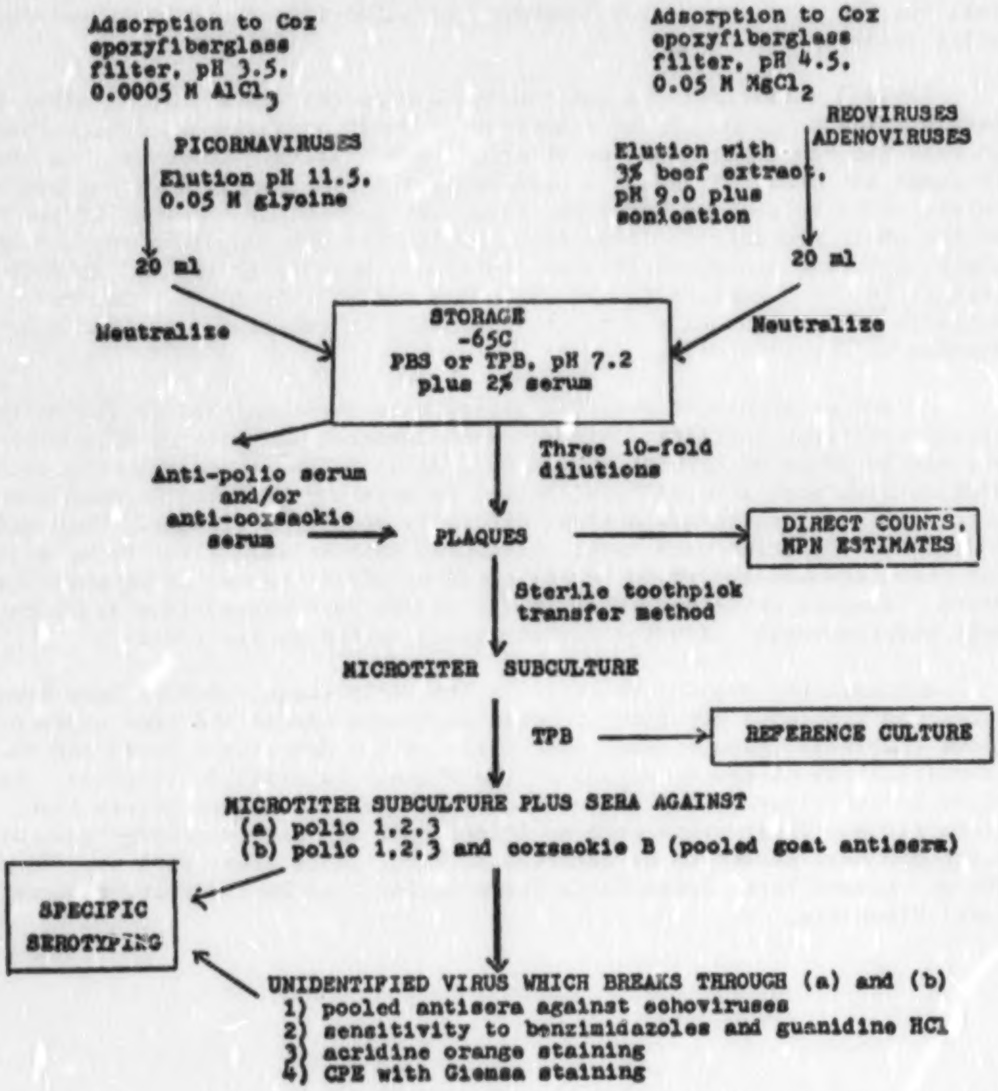
Adsorption to Cox epoxyfiberglass filter, pH 3.5, 0.0005 M AlCl3
PICORNAVIRUSES
Elution pH 11.5, 0.05 M glycine
20 ml
Adsorption to Cox epoxyfiberglass filter, pH 4.5, 0.05 M MgCl2
REOVIRUSES
ADENOVIRUSES
Elution with 3% beef extract, pH 9.0 plus sonication
20 ml
Neutralize
Neutralize
STORAGE -65C PBS or TPB, pH 7.2 plus 2% serum
Three 10-fold dilutions
Anti-polio serum and/or anti-coxsackie serum
PLAQUES
DIRECT COUNTS, MPN ESTIMATES
Sterile toothpick transfer method
MICROTITER SUBCULTURE
TPB
REFERENCE CULTURE
MICROTITER SUBCULTURE PLUS SERA AGAINST
(a) polio 1,2,3
(b) polio 1,2,3 and coxsackie B (pooled goat antisera)
SPECIFIC SEROTYPING
UNIDENTIFIED VIRUS WHICH BREAKS THROUGH (a) and (b)
1) pooled antisera against echoviruses
2) sensitivity to benzimidazoles and guanidine HCl
3) acridine orange staining
4) CPE with Giemsa staining

Table 1. Relative survival of enteroviruses during shipment. Following collection the water samples were maintained at ambient temperatures for 10 hours and at 4C for 12 to 15 hours.

				Per cent survivors after dilution and shipment in:			
Sample & Number	Virus	TPB		Creek Water Gwynns Falls (Site D)		Sewage Back River Treatment Plant (Site A)	
7/29	Polio-1	100	pH 7.3	20	pH 7.0	80	pH 6.0
8/5	Polio-1			5	pH 6.8		
9/17	Polio-1	≈100	pH 7.3	13	pH 6.9	≈100	
9/24	Polio-1			36	pH 6.9		
		160		16	pH 6.9		
10/29	Polio-1	69	pH 7.3	50	pH 7.3		
1/14 (#27)	Polio-1	100	pH 7.3				
1/20 (#30)	Polio-1	110	pH 7.3	70	pH 7.25	81	pH 6.9
9/24	Coxsackie B3	66	pH 7.3	33	pH 6.9		
1/20 (#30)	Coxsackie B3	90	pH 7.3	82	pH 7.25	95	
9/24	Echo-7	88	pH 7.3	54	pH 6.9		
1/20 (#30)	Echo-7	99	pH 7.3	77	pH 7.25	69	pH 6.9

Table 2. Survival of poliovirus 1 supplied as a tissue culture supernatant, diluted 1:10 in Gwynns Falls Creek water and shipped to Syracuse, N.Y. The tissue culture maintenance medium contained Eagle's minimum essential medium (E-MEM) with 5 per cent fetal calf serum.

Virus treatment	Hours	Relative titers (PFU/ml)	Per cent survival
TC virus, thawed and titered	0	6×10^6	100
TC virus, diluted in creek water and shipped to Syracuse	10	7.9×10^5	13
TC virus diluted in creek water, shipped and stored at 4C	216	8.3×10^5	14

Table 3. Summary of natural occurrence of viruses in untreated water sources. Estimates are based on MPN values for 8 tubes inoculated with each of three successive 10-fold dilutions.

Water	Source	No. Samples	Total no. positive samples	No. positive samples after add'n anti-Polio serum	Average sample (liters)
Sewage	Syracuse[c]	11	11	6	4 to 260
Sewage	Baltimore[a,c]	4	4	4	19
Storm	Syracuse	14	8	4	111 to 193
Storm	Baltimore[a]	16	7	5	19
Creek[b]	Baltimore[a]	10	6	0	19

[a] Through 2/24/74

[b] Dry weather flow

[c] Treatment plant influent

Table 4. Recovery of viruses from Syracuse storm overflows.

Storm Sample	Sample gal.	Polio	PFU/gallon Coxsackie	Echo	Other
raw	20	0	0	--	0
chlorinated[a]	20	43	0	--	0
raw	45	230	7	--	9
chlorinated	45	181	38	--	21
raw (early)	51.1	7	1	1	0
chlorinated (early)	44.3	0	0	0	1
raw (early)	52.2	2	0	0	0
chlorinated (early)	30	6	0	0	0
raw (late)	52.2	0	0	0	0
chlorinated (late)	30	0	0	0	0
raw	50.8	0	0	0	0
chlorinated	52.1	1	0	15	0
raw	51.4	22	0	0	0
chlorinated	50.9	0	0	0	0

[a] chlorine dioxide

Table 5. Recovery of viruses from Baltimore storm overflows.

	Water	Place	MPN/gallon 1/14	 1/21	 2/11	 2/24
A	Dry Flow	Back River Treatment Plant		375		
B		Herring Run		8		
C		Jones Falls		70		
D		Gwynns Falls		3		
F	Storm Overflow	Stoney Run	0		0	1
G		Western Run	0		$2x10^4$	0
H		Howard Park	1		2	0
K		Jones Falls (Storm outlet)	0		43	0
L		Bush Street	5			11
M		Northwood	0			0

THE ENHANCEMENT OF HIGH-RATE DISINFECTION BY THE SEQUENTIAL ADDITION OF CHLORINE AND CHLORINE DIOXIDE

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ABSTRACT

The magnitude of combined sewer overflows (CSO) that occur within urban areas limits the use of conventional disinfection techniques that necessitate large facilities. Point-source treatment of CSO by high-rate application of 25 mg/l chlorine (Cl_2) or 12 mg/l chlorine dioxide (ClO_2) reduced indicator bacterial and viral counts to levels acceptable for discharge to recreational waters. When added sequentially at 15 to 30 second intervals, 8 mg/l Cl_2 followed by 2 mg/l ClO_2 achieved lower bacterial counts after two minutes contact time than would be predicted from corresponding single-stage disinfection results. This effect may be due to an interaction between the two disinfectants rather than a reduction in demand for ClO_2 caused by the prior addition of Cl_2. It is hypothesized that the more potent disinfectant, ClO_2, is regenerated by the reaction of Cl_2 with chlorite (ClO_2), the decomposition product of ClO_2.

Introduction

Combined sewer overflows (CSO) remain one of the most neglected sources of microbial contamination of surface waters. Although intermittent in nature, the volumes of these overflows are sufficient to raise the indicator bacterial counts in receiving waters to levels that preclude contact recreation. The occurrence of most CSO within highly developed urban areas, where land is often unavailable or expensive, restricts the use of conventional treatment techniques that require long detention times and large facilities. Several methods of high-rate point-source treatment of CSO were studied in an effort to circumvent this restriction. The facilities, which are described in detail elsewhere (Moffa et al., 1975), include fine-mesh screening or swirl concentration to reduce suspended solids followed by disinfection with chlorine (Cl_2) or chlorine dioxide (ClO_2) to reduce microbial organisms.

Cl_2 has been found to give satisfactory disinfection in similar applications (Glover and Herbert, 1973). However, concern about the generation of possible carcinogens through chlorination (Dowty et al., 1975), and the cost and availability of Cl_2 would indicate the desirability of an alternate disinfectant. ClO_2 has shown potential in previous limited applications and was investigated as an alternative. Although many reactions for the manufacture of ClO_2 use Cl_2 as a starting material, at least one method of generation (Callerame, 1973) requires only other common chemicals.

The evaluation was conducted in two parts. First a series of bench-scale studies was performed to determine the approximate dosages of Cl_2 and ClO_2 that would reduce microbial counts to acceptable levels. The second phase consisted of verification of these findings by operation of two full-scale prototype treatment facilities for CSO.

Methods

The bench-scale studies were conducted according to the flow charts given in Figures 1 and 2. The disinfectants, Cl_2 and ClO_2, were applied individually and sequentially (single-stage and two-stage studies, respectively) to portions of a simulated combined sewer overflow (SCSO). The SCSO was a mixture of equal parts distilled deionized water and influent to the Onondaga County Metropolitan Treatment Plant and was chosen for ease and consistency of comparisons to be carried out over several months. Samples for bacterial and viral analysis were collected in bottles containing an amount of sodium thiosulfate sufficient to halt the disinfection by either Cl_2 or ClO_2. Bench-scale screening was performed by passing the samples through a screen with a 23 micron pore diameter.

The site plans for the prototype treatment facilities are shown in Figures 3 and 4 for Maltbie Street and Figure 5 for West Newell Street. The Maltbie Street facility was designed around the concept of high-rate, fine-mesh screening followed by high-rate disinfection. Three parallel screening units, a Crane Microstrainer (Crane Co., King of Prussia, Pa.) with a screen aperture of 23 microns, a Zurn Micro-Matic (Zurn Industries, Inc., Erie, Pa.) with a screen aperture of 71 microns and a Sweco Wastewater Concentrator (Southwestern Engineering Co., Massilon, Ohio) with a screen aperture of 105 microns, can each receive equal flow up to a maximum of 219 l/sec (5 mgd) flow. The effluent from each unit can be disinfected with either Cl_2 or ClO_2 for a contact time of up to two minutes. A proportioning weir was constructed so the contact time could be held constant with different flows.

The West Newell Street facility was designed around the swirl concentrator conceived in England (Smisson, 1967) and further developed by the U. S. Environmental Protection Agency (Field, 1973). The influent to the swirl concentrator may reach a maximum of 400 l/sec (8.9 mgd) and any flow in excess of 66 l/sec (1.5 mgd) will overflow the regulating weir. The outfall pipe for this overflow serves as the contact chamber for disinfection so the contact time will vary with flow up to one minute.

Samples were collected at the designated locations in Figures 4 and 5 with refrigerated sequential sampler (Sigmamotor, Inc., Middleport, N.Y.). For disinfected samples, sodium thiosulfate was added to each of the bottles before sample collection. The standard bacterial indicators of water quality, total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) were enumerated using the membrane filter (MF) technique (Standard Methods, 1971) with three replicates for each sample. Although there is some question surrounding the validity of the MF technique in chlorinated waste-waters, periodic correlation with the most probable number (MPN) technique established the reliability of the MF technique in chlorinated CSO for this study. All samples were blended for approximately six seconds at approximately 3000 rpm in order to enumerate any bacteria that may have been harbored within suspended matter and grease particles. The effects of disinfection were also measured by enumeration of two bacterial viruses, f_2 and ØX174 and one enteric virus, Poliovirus Sabin Type 1 (Polio-1). Due to the relatively low natural levels of virus in wastewater, the samples were seeded with a known concentration of test virus prior to disinfection. The viruses were preconcentrated on a membrane filter before enumeration by the plaque assay method (Dulbecco and Vost, 1954).

Cl_2 was obtained as a five percent solution of sodium hypochlorite in water. Standardization of stock solutions and determinations of residual Cl_2 were accomplished using the thiosulfate and DPD methods (Standard Methods, 1971). ClO_2 was generated on a bench-scale by acidification of a solution of sodium chlorite (Standard Methods, 1971) and on a full-scale by the nitrosyl chloride method (Callerame, 1973). The measurement of ClO_2 concentrations was performed by the DPD technique and the use of electron spin resonance (esr) which has been advocated as a primary standard method for the analysis of ClO_2 (Murphy and Tifft, 1973).

Results and Discussion

The results of single-stage disinfection of bacteria in SCSO with Cl_2 and ClO_2 are shown in Tables 1 through 4. Since each trial was run on a separate batch of SCSO, the initial bacterial counts varied by an order of magnitude or more. Consequently, the results were converted to fraction survivors in order to average the values. Two of the trials were run on screened SCSO (23 micron aperture) to determine the effects of screening, but the variations in duplicate trials were of the same magnitude as the difference between screened and unscreened trials. The variations in fraction survivors from the reported averages ranged from 10 to 50 percent. The repeated measurement of bacterial populations in CSO shows an approximate average maximum value of 5,000,000 TC/100 ml (Moffa et al., 1975). The general guidelines for maximum bacteria levels in the discharge are 1,000 TC/100 ml and a fraction survivors of 0.0002 would correspond to the desired effluent population under adverse conditions. The results indicate that these reductions can be obtained with 25 mg/l Cl_2 or 12 mg/l ClO_2 within 120 seconds. The results also show that FS may be more resistant to disinfection and perhaps a more reliable bacterial indicator.

The results of single-stage high-rate bench-scale disinfection of viruses with Cl_2 and ClO_2 are summarized in Table 5.

Table 1

Single-Stage Disinfection of Total Coliform in SCSO with Cl_2[a]

Contact Time (seconds)	Cl_2 Dosage mg/l 0	4	8	12	16	20	25
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00
30	0.86	0.32	0.83	0.10	0.13	2.11	0.0067
60	1.10	0.63	0.26	0.049	0.037	0.0020	0.0030
120	1.28	0.22	0.19	0.043	0.034	0.0011	0.00021
180	1.00	0.88	0.18	0.013	0.00039	0.00012	0.00093
300	1.06	0.44	0.079	0.0052	0.00059	0.000057	0.00087

[a] Values in fraction survivors

Table 2

Single-Stage Disinfection of Fecal Strep in SCSO With Cl_2[a]

Contact Time (seconds)	0	4	8	12	16	20	25
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00
30	0.99	0.77	0.78	0.44	0.27	0.17	0.056
60	1.14	0.77	0.47	0.30	0.26	0.10	0.009
120	0.89	0.68	0.23	0.18	0.11	0.02	0.006
180	0.93	0.58	0.22	0.10	0.06	0.02	0.001
300	0.98	0.44	0.08	0.05	0.03	0.01	0.002

[a] Values in fraction survivors

Table 3

Single-Stage Disinfection of Total

Coliform in SCSO With ClO_2[a]

Contact Time (seconds)	ClO_2 Disage (mg/l)						
	0	4	8	12	16	20	25
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00
30	0.92	0.199	0.018	0.0060	0.0049	0.0121	0.0017
60	0.80	0.120	0.014	0.003	0.0017	0.0007	0.0009
120	1.07	0.088	0.010	0.0008	0.0012	0.0005	0.0005
180	0.74	0.024	0.001	0.0002	0.0001	0.0001	0.0001
300	0.90	0.127	0.012	0.0009	0.0012	0.0002	0.0003

[a] Values in fraction survivors

Table 4

Single-Stage Disinfection of Fecal Strep in SCSO With ClO_2[a]

Contact Time (seconds)	ClO_2 Dosage (mg/l)						
	0	4	8	12	16	20	25
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00
30	0.98	0.30	0.064	0.020	0.010	0.0026	0.0034
60	0.97	0.50	0.056	0.017	0.0026	0.0022	0.0022
120	0.83	0.21	0.041	0.016	0.0016	0.0010	0.0006
180	0.88	0.13	0.0016	0.0010	0.0002	0.0001	0.0001
300	0.95	0.25	0.0054	0.0016	0.0009	0.0002	0.0001

[a] Values in fraction survivors

Table 5

Disinfection of Viruses

Virus	Disinfectant	Dosage mg/l	Contact Time (seconds)	Log Red
ØX174	Cl_2	20	120	4.7
ØX174	Cl_2	25	120	5.0
ØX174	ClO_2	12	30	5.4
Polio-1	ClO_2	8	30	2.2
Polio-1	ClO_2	12	120	4.0
Polio-1	ClO_2	16	120	5.0
f2	Cl_2	20	120	3.0
f2	ClO_2	4	120	2.8
f2	ClO_2	7	120	3.6
f2	ClO_2	9	120	4.5

The values are reported in terms of logarithm reductions for the following reasons. Natural virus levels in sewage and overflows are generally unknown, but it is assumed that the values would rarely exceed 1 X 10^5 pfu (plaque forming unit)/ml. Thus, a reduction of five logarithms would bring the virus levels to 1 X 10^0 pfu/ml or less. This target is completely arbitrary and should not be taken as a viral standard because the ingestion of only one virus particle may be an infectious dose. Present methods of viral enumeration do not permit the rapid detection of counts below 1 X 10^3 pfu/ml in wastewaters. Therefore, in order to facilitate the experiments, the samples were seeded to give initial levels of 1 X 10^8 pfu/ml. It was assumed that a reduction from 10^8 to 10^3 pfu/ml can be accomplished under the same disinfectant conditions as a reduction from 10^5 to 10^0 pfu/ml. This is based on the fact that in these ranges the number of disinfectant-demanding organisms is negligible compared to the number of disinfectant molecules. Therefore, the rate of kill is a function of disinfectant concentration and contact time but not viral population. These results indicate that 25 mg/l Cl_2 and 12 mg/l ClO_2 would give satisfactory virus kills in 120 seconds. The virus studies are discussed in greater detail elsewhere (Smith and McVea, 1975).

The next step involved verification of these findings in the operation of the full-scale prototype treatment facilities. Table 6 summarizes the work that was completed in 1974. For

Table 6

Disinfection of CSO

Facility	Overflow		Flow Rate (mgd)	Disin-fectant	Dosage (mg/l)	Bacterial Reduction Fraction Survivors		
	Date	Time				TC	FC	FS
Maltbie Sweco	6/10/74	20:00	1.88	ClO_2	14.6	0.32	0.30	--
	6/10/74	20:20	1.88	ClO_2	14.6	0.02	--	--
	6/14/74	11:30	0.75	ClO_2	7.5	0.0027	0.012	0.017
	6/14/74	10:30	0.62	ClO_2	8.5	0.010	0.024	0.015
	7/29/74	18:10	3.40	ClO_2	7.0	--	0.011	--
	7/29/74	18:50	3.40	ClO_2	7.0	--	0.012	--
	7/29/74	19:30	3.40	ClO_2	7.0	--	0.012	--
Newell Swirl	9/3/74	9:10	0.50	ClO_2	8.0	0.00084	0.0093	--
	9/3/74	11:00	0.50	ClO_2	8.0	0.030	0.0067	--
	9/3/74	11:30	0.50	ClO_2	8.0	0.0004	0.028	--
Maltbie Zurn	9/21/74	17:30	1.00	ClO_2	12.2	0.097	--	--

all trials the contact time was approximately one minute. The data shows that the target disinfection, as defined previously in this paper, is possible.

The effect of one disinfectant (Cl_2) followed by a second (ClO_2) after an interval of 15 to 30 seconds (two-stage disinfection) is presented in Table 7.

Table 7

Two-Stage Disinfection of Bacteria in SCSO

Cl_2 Dosage (mg/l)	ClO_2 Dosage (mg/l)	Interval (Seconds)	Fraction Survivors at 120 Seconds		
			TC	FS	FC
4	2	15	0.015	0.039	--
4	2	30	0.034	0.1[illegible]	--
4	4	15	0.032	0.055	--
4	4	30	0.0050	0.031	--
8	2	15	0.00059	--	0.00074
8	2	30	0.00045	--	0.0011
8	4	15	0.00015	0.00099	0.000030
8	4	30	0.00010	0.00059	0.000040

Varying the interval between the addition of each disinfectant from 15 to 30 seconds did not affect the rate of kill, presumably an effect of the rate of mixing. In order to determine if disinfection had been enhanced beyond the additive effects of the different agents, the log reductions were compared. Table 8 includes the log reductions in TC accomplished by 4 mg/l and 8 mg/l Cl_2, and 2 mg/l and 4 mg/l ClO_2 as determined in the single-stage studies (from Tables 1 and 3, respectively). The sums of the reductions were compared to the observed log reductions in the two-stage studies for the corresponding dosages in Table 9.

Table 8

Average TC Reductions for Single-Stage Disinfection

Disinfectant	Dosage (mg/l)	Time (seconds)	Log Reduction
Cl_2	4	120	0.66
Cl_2	8	120	0.72
ClO_2	2	90	0.50[a]
ClO_2	4	90	0.99[a]

[a] Interpolated Values

Table 9

Average TC Reductions for Two-Stage Disinfection

First Disinfectant	Dosage (mg/l)	Second Disinfectant	Dosage (mg/l)	Log Reduction at 120 Seconds	
				Predicted	Observed
Cl_2	4	ClO_2	2	1.16	1.60
Cl_2	4	ClO_2	4	1.65	1.80
Cl_2	8	ClO_2	2	1.22	3.28
Cl_2	8	ClO_2	4	1.71	3.92

At low reductions there were small differences between observed and predicted values, most likely because of difficulties in measuring small differences in large numbers. As the reliability in reported differences increased, the enhancement of disinfection became more easily discernible.

It was first thought that Cl_2 might precondition the SCSO by reducing the demand for ClO_2. To test this hypothesis, a sample of SCSO was split into two portions for determination of Cl_2 and ClO_2 demands. The sample for which the Cl_2 demand had been satisfied was then dechlorinated with thiosulfate. The ClO_2 demand of this sample was found to be about 80% of the ClO_2 demand of the original sample. Thus, some other mechanism must be responsible for the enhanced disinfection.

It is possible that the two disinfectants could interact through the following mechanism. One of the reactions for the preparation of ClO_2 involves the addition of Cl_2 to a solution of ClO_2.

$$H^+ + HOCl + 2\ NaClO_2 \longrightarrow 2\ ClO_2 + NaCl + Na^+ + H_2O$$

After the ClO_2 has been oxidized to ClO_2^- in the SCSO, the free Cl_2 also present might reduce the ClO_2^- back to ClO_2. This process would prolong the existence of the more potent disinfectant, ClO_2, and thus enhance disinfection beyond that expected by the sum of the respective concentrations of Cl_2 and ClO_2. It might be argued that the reduction in free Cl_2 would negate the benefit of the regenerated ClO_2 but it has been shown that ClO_2 is a more powerful disinfectant when taken on a weight basis. In essence, two-stage disinfection allows the substitution of 2 mg/l ClO_2 for 17 mg/l Cl_2, a ratio of

8.5 to 1. Current figures show that the cost of one pound of ClO_2 is three to four times the cost of one pound of Cl_2. Thus, two-stage application of Cl_2 and ClO_2 may result in significant savings.

CONCLUSIONS

The economic and environmental objections to the use of chlorine for terminal wastewater disinfection have led to the investigation of alternative disinfectants. Twelve mg/l of chlorine dioxide has given the same bacterial and viral kills as twenty-five mg/l chlorine in two minutes contact time. This is especially pertinent to disinfection of combined sewer overflows. The effects of chlorite ion, the by-product of disinfection with ClO_2, remain to be investigated. The possibility of using both disinfectants in combination (8 mg/l Cl_2 and 2 mg/l ClO_2) may be a way to increase efficiency and reduce operating expenses.

ACKNOWLEDGEMENTS

The virus studies were conducted by James E. Smith of the Biological Research Laboratories of Syracuse University. The assistance of John M, Karanik, Project Officer for the Division of Sanitation and Drainage, Department of Public Works, County of Onondaga, New York, is acknowledged. This work was co-sponsored by the County of Onondaga and the U. S. Environmental Protection Agency, Grant No. S-802400.

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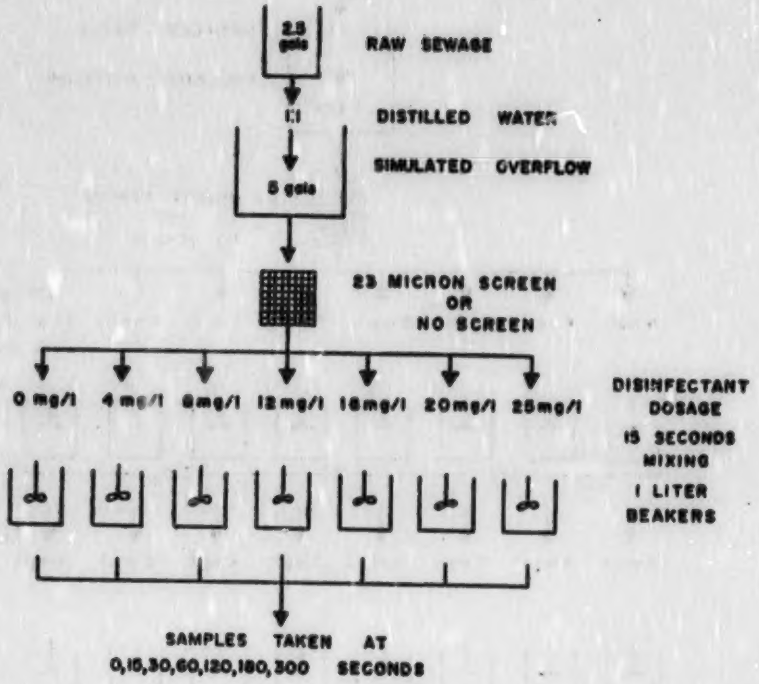
2.5 gals
RAW SEWAGE
1:1
DISTILLED WATER
SIMULATED OVERFLOW
5 gals
23 MICRON SCREEN
OR
NO SCREEN
0 mg/l
4 mg/l
8 mg/l
12 mg/l
16 mg/l
20 mg/l
25 mg/l
DISINFECTANT DOSAGE
15 SECONDS MIXING
1 LITER BEAKERS
SAMPLES TAKEN AT
0,15,30,60,120,180,300 SECONDS

FIGURE 1. OUTLINE FOR SINGLE-STAGE DISINFECTION

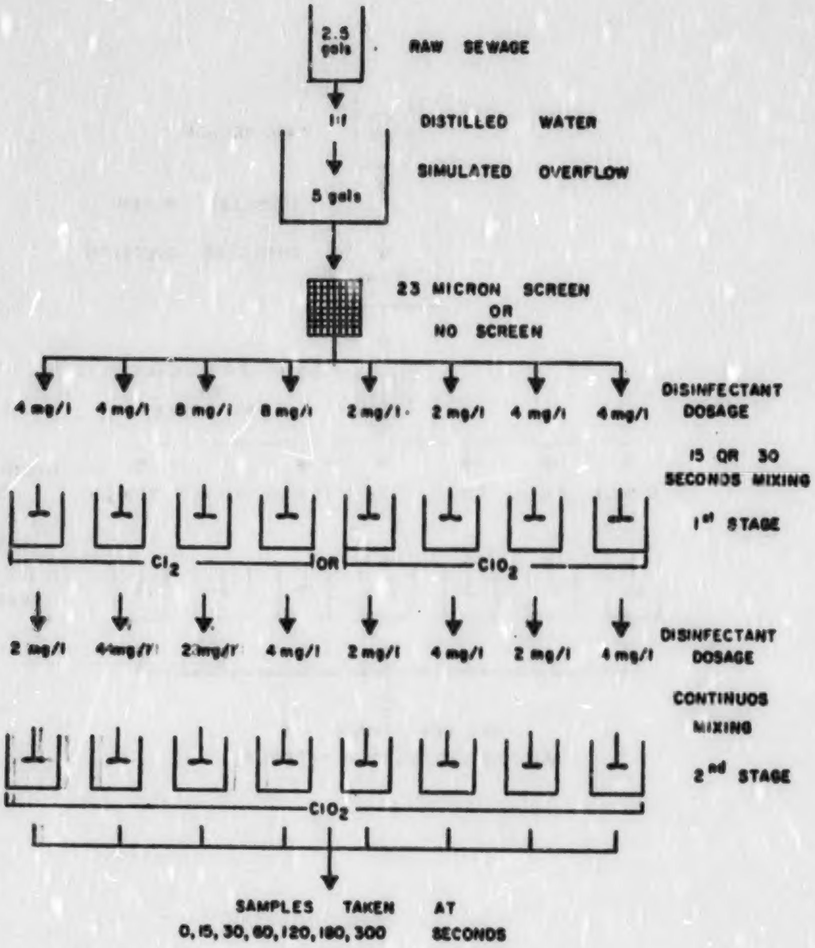
2.5 gals
RAW SEWAGE
1:1
DISTILLED WATER
SIMULATED OVERFLOW
5 gals
23 MICRON SCREEN
OR
NO SCREEN
4 mg/l
4 mg/l
8 mg/l
8 mg/l
2 mg/l
2 mg/l
4 mg/l
4 mg/l
DISINFECTANT DOSAGE
15 OR 30 SECONDS MIXING
1st STAGE
Cl2
OR
ClO2
2 mg/l
4 mg/l
2 mg/l
4 mg/l
2 mg/l
4 mg/l
2 mg/l
4 mg/l
DISINFECTANT DOSAGE
CONTINUOS MIXING
2nd STAGE
ClO2
SAMPLES TAKEN AT
0, 15, 30, 60, 120, 180, 300 SECONDS

FIGURE 2. OUTLINE FOR TWO-STAGE DISINFECTION

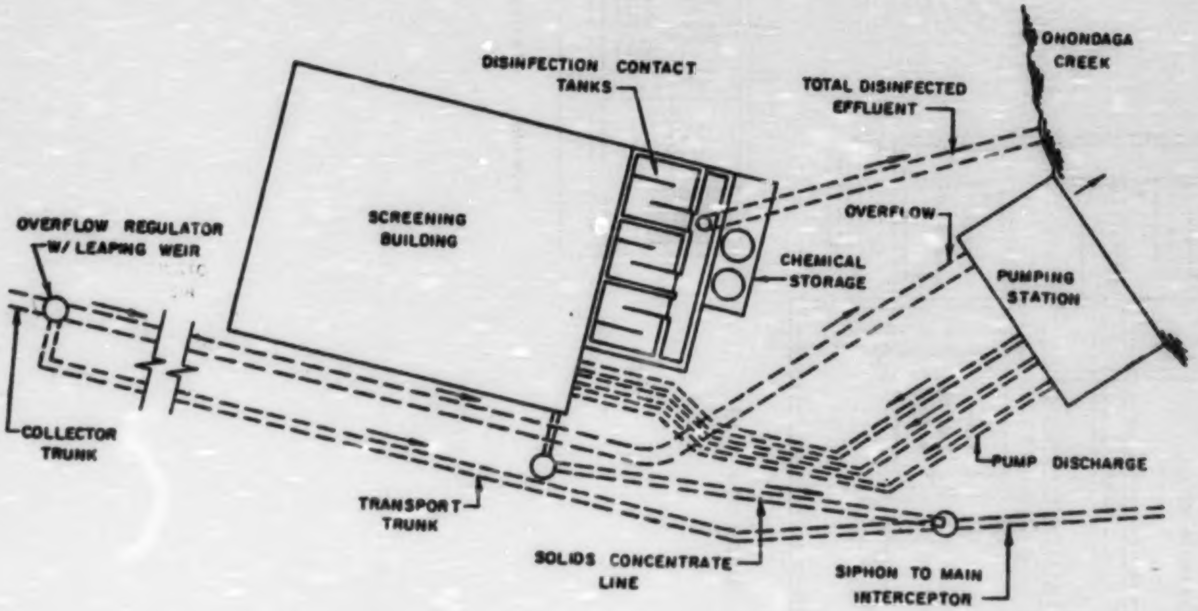
DISINFECTION CONTACT TANKS
ONONDAGA CREEK
TOTAL DISINFECTED EFFLUENT
SCREENING BUILDING
OVERFLOW
OVERFLOW REGULATOR W/ LEAPING WEIR
CHEMICAL STORAGE
PUMPING STATION
COLLECTOR TRUNK
PUMP DISCHARGE
TRANSPORT TRUNK
SOLIDS CONCENTRATE LINE
SIPHON TO MAIN INTERCEPTOR

FIGURE 3. MALTBIE STREET SITE PLAN

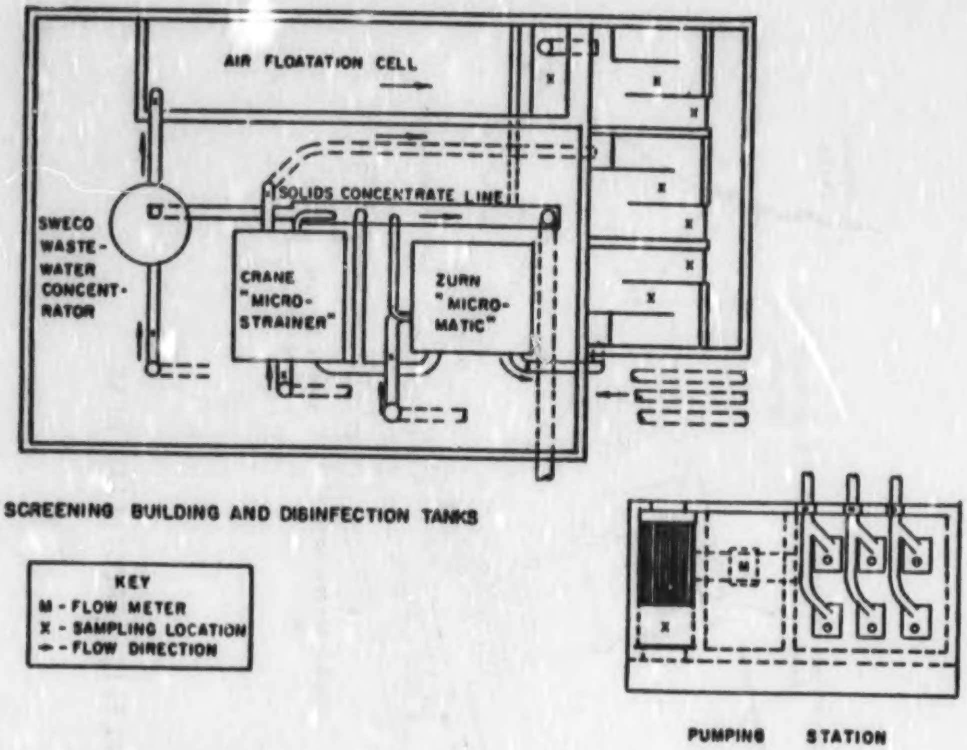
AIR FLOATATION CELL
SOLIDS CONCENTRATE LINE
SWECO WASTE-WATER CONCENTRATOR
CRANE "MICRO-STRAINER"
ZURN "MICRO-MATIC"
X
SCREENING BUILDING AND DISINFECTION TANKS
KEY
M - FLOW METER
X - SAMPLING LOCATION
← - FLOW DIRECTION
M
PUMPING STATION

FIGURE 4. MALTBIE STREET PROCESS ORIENTATION

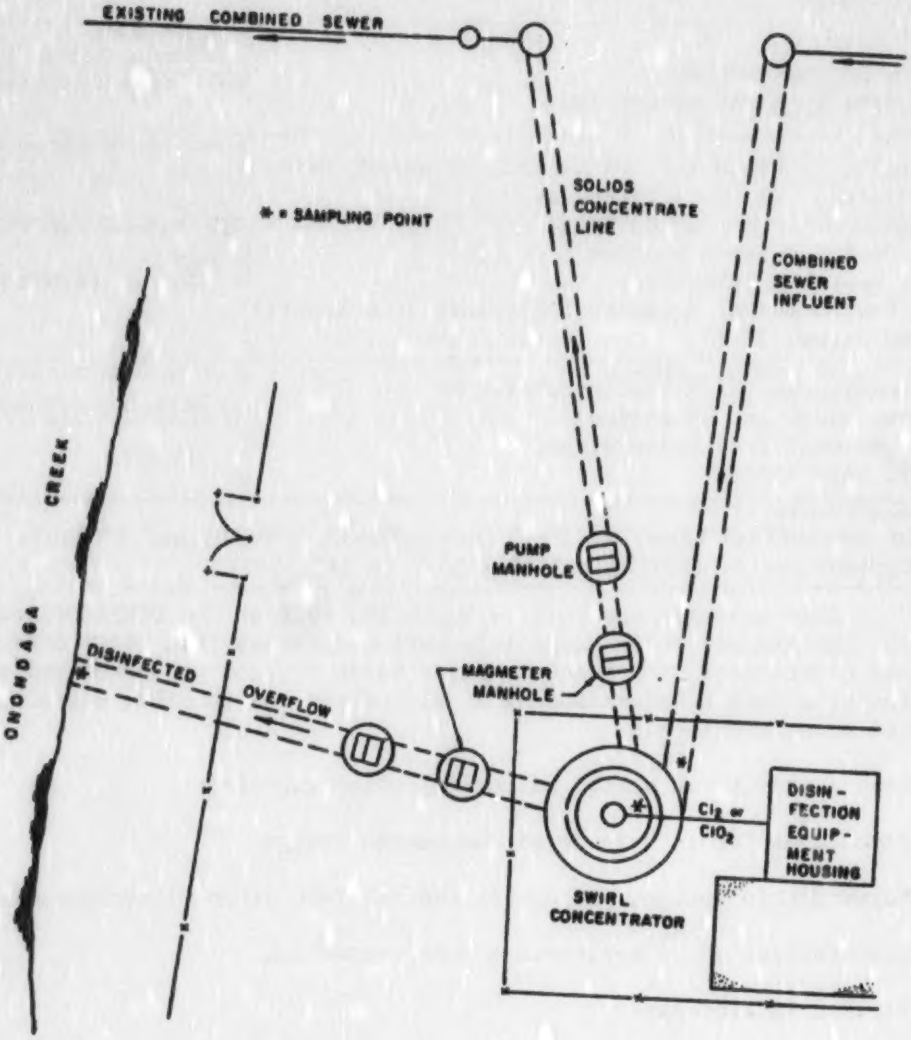
EXISTING COMBINED SEWER
* = SAMPLING POINT
SOLIDS CONCENTRATE LINE
COMBINED SEWER INFLUENT
ONONDAGA CREEK
PUMP MANHOLE
MAGMETER MANHOLE
DISINFECTED OVERFLOW
DISIN-FECTION EQUIP-MENT HOUSING
Cl2 or ClO2
SWIRL CONCENTRATOR

FIGURE 5. WEST NEWELL STREET SITE PLAN